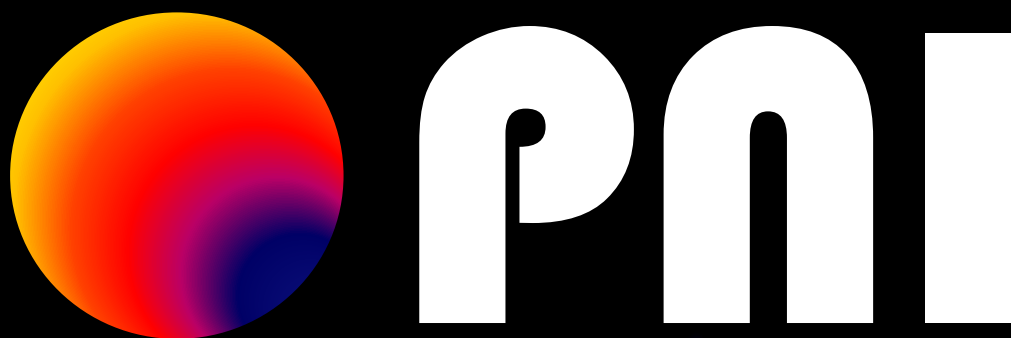
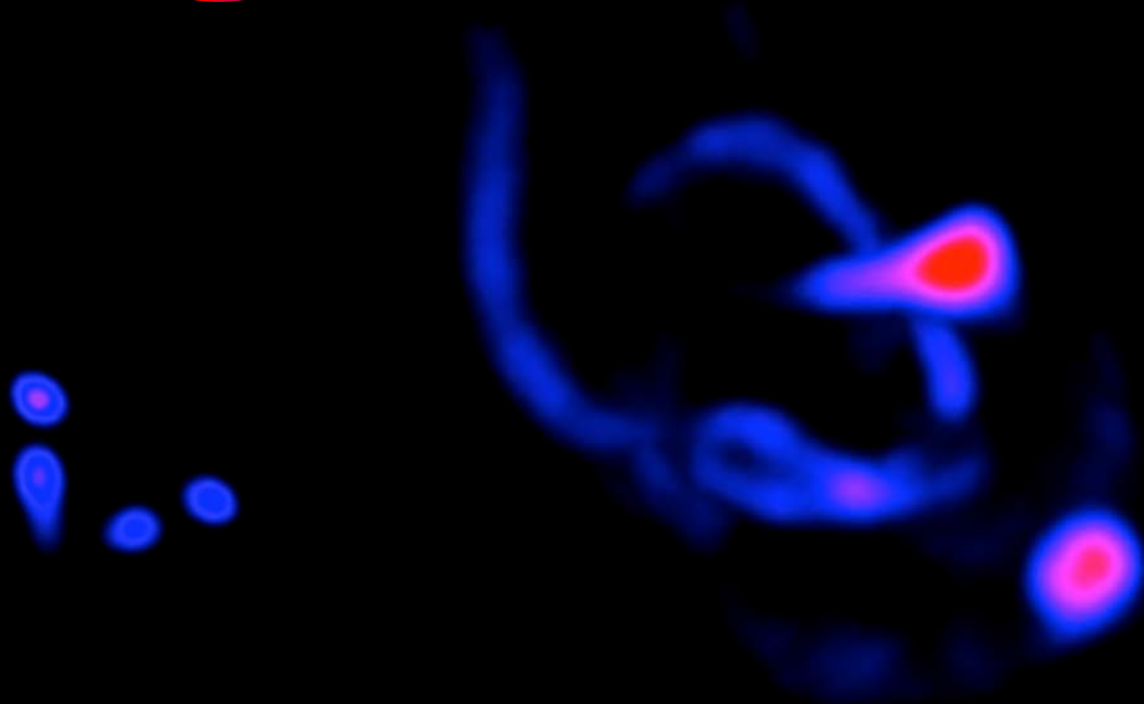




Sixth Symposium on
Preclinical Nuclear Imaging
Cambridge
02 November 2026



UNIVERSITY OF
CAMBRIDGE

TABLE OF CONTENTS

	Page
GREETINGS	2
PROGRAMME	4
ABSTRACTS	
Oral communications (O 01 – O 11)	7
Keynote lecture (O 12)	26
Poster presentations (P 01 – P 23)	28
SERVICE SECTION	
Internet access	72
PNI TEAM	
PNI organising committee	73
ENDORSEMENT AND SPONSORSHIP	
Endorsement	74
Sponsors	74

GREETINGS

DEAR COLLEAGUES AND FRIENDS

Welcome to the Sixth Symposium on Preclinical Nuclear Imaging (PNI)!

On behalf of the organising committee, it is our great pleasure to welcome you to Cambridge for the 6th Symposium on Preclinical Nuclear Imaging (PNI 2026).

We are delighted to host this year's meeting on the Cambridge Biomedical Campus, one of Europe's leading centres for biomedical research,



bringing together researchers from academia, healthcare, industry and technology to accelerate scientific discovery and translation for patient benefit.

Cambridge has a long association with positron emission tomography (PET) and molecular imaging research. Since the opening of the Wolfson Brain Imaging Centre (WBIC) in 1996, the University has established itself as a leading centre for PET research, advancing radiochemistry, tracer development, imaging methodology and translational neuroscience.

Today, preclinical PET research spans multiple departments and institutes across Cambridge. The Molecular Imaging Chemistry Laboratory (MICL), established in 2008 under the leadership of Professor Franklin Aigbirhio, has played a central role in developing novel radiotracers and radiochemical methodologies. Within the Department of Radiology, the recently established Preclinical Imaging Research Laboratory (PIRL) provides PET, CT and MRI imaging expertise and research support to investigators across the University of Cambridge, neighbouring academic and healthcare institutions, and pharmaceutical and biotechnology partners. The Cancer Research UK Cambridge Institute maintains extensive preclinical PET, SPECT, CT and MRI capabilities supporting cancer biology, therapy development and translational oncology. Beyond the Biomedical Campus, PET imaging research also extends across the wider University, including the West Cambridge site, where imaging is applied to fundamental questions in neuroscience and brain function. Looking ahead, Cambridge's planned installation of a total-body PET scanner in 2027 represents an exciting new chapter in this translational pathway, creating unique opportunities to connect advances in preclinical whole-body imaging with next-generation human total-body PET studies. Together, these diverse research activities exemplify the innovation that continues to drive molecular imaging forward, a theme that is reflected throughout the outstanding abstracts presented at PNI 2026.

Abstracts from leading groups across the UK demonstrate the breadth and innovation of the preclinical imaging field. From novel radiotracers and theranostic agents to advances in quantitative imaging, molecular radiotherapy, infection imaging, neuroscience, oncology and cardiometabolic research, the work presented here highlights the central role of molecular imaging in bridging fundamental biology and clinical translation. We are particularly encouraged by the strong representation of early-career researchers, interdisciplinary collaborations, and contributions from both academic and industrial scientists.

A special welcome is extended to our keynote speaker, Professor Peter J H Scott from the University of Michigan. His keynote lecture perfectly captures the innovation and forward-thinking spirit of the preclinical imaging community. As artificial intelligence increasingly influences scientific discovery, we look forward to hearing how these emerging technologies may help reshape the future of PET tracer development and molecular imaging research.

We are particularly grateful to our invited speakers, abstract presenters, session chairs, pharmaceutical and industry researchers, and delegates, whose contributions make this meeting possible. We are also pleased to welcome students and early-career researchers, whose enthusiasm and innovation will help shape the future of our field.

We are deeply grateful to our sponsors for their generous support of PNI 2026. Their commitment to advancing molecular imaging research, innovation and scientific collaboration has been instrumental in making this symposium possible. We encourage delegates to visit the exhibition stands, engage with our industry partners, and learn about the latest technologies and opportunities shaping the future of preclinical nuclear imaging.

We also gratefully acknowledge the European Society for Molecular Imaging (ESMI) for endorsing PNI 2026 and for sponsoring the prize for outstanding early career research, recognising excellence and innovation within our community.

We hope that, in addition to the scientific programme, you will take the opportunity to engage with colleagues old and new and develop collaborations that extend beyond today's meeting.

Thank you for joining us, and we wish you an enjoyable and scientifically stimulating symposium.



Dr Nisha Kuzhupilly Ramakrishnan



Dr Flaviu Bulat

PROGRAMME

INTRODUCTION

Chair *Dr Nisha Kuzhupilly Ramakrishnan*

9:30 Registration

10:00 Welcome address

10:05 ESMI and youngESMI

SESSION 1 DISCOVERY

Chair *Dr Kerstin Sander*

10:15 O 01 Prof Graeme Stasiuk

Advancing chelator design to enhance preclinical imaging for metal-based radiotracers

10:45 O 02 Dr Isabella Ogilvy

^{18}F -Radiopharmaceutical diversification enabled by deaminative cross-electrophile couplings

10:55 O 03 Mr Allen Drews

Quantitative SPECT: limits of dynamic 3D mouse thyroid imaging with $^{99\text{m}}\text{Tc}$ -pertechnetate

11:05 **Coffee break**

SESSION 2 DEVELOPMENT

Chair *Prof Tammy Kalber*

11:35 O 04 Dr Gemma Mudd

Bicycle radioconjugates: a new modality for molecular imaging of novel targets

12:05 O 05 Dr Andrea Emanuelli

Imaging tumour antioxidant capacity with the novel xCT-targeted PET radiotracer (*S*)-4-(4- ^{18}F -fluorobenzyl)-L-glutamate [^{18}F]FSBG

- 12:15 **O 06** Dr Tia Gibson
Polysarcosine star dendrimers: an efficient drug delivery system to tumours with minimal accelerated blood clearance
- 12:25 **O 07** Dr Francis Man
 $^{225}\text{Ac}/^{89}\text{Zr}$ -hNd2: novel theranostic agents targeting MUC5AC-positive cancers

SESSION 3

Chairs *Dr Flaviu Bulat and Dr Joshua Kaggie*

- 12:35 Poster pitch flash presentations
- P 22** Mr Allen Drews
Phantom characterization of quantification limits in ^{18}F and $^{99\text{m}}\text{Tc}$ dual-isotope SPECT imaging
- P 13** Ms Anishaa Balaji
Development of an in vivo traceable orthotopic rat breast cancer model for longitudinal assessment of EGFR-targeted therapy
- P 05** Mr Shinong Zeng
Synthesis of fluorinated *N*-acylethanolamine acid amidase (NAAA) inhibitors to enable the development of a radiotracer targeting NAAA
- P 07** Ms Rosie Lewis
Supramolecular radiotheranostics in non-small cell lung cancer
- P 18** Dr Zhiping Feng
Multimodal imaging of heart-brain alterations in a mouse model of diabetes
- P 12** Dr Abdullah Alyamani
Dynamic ^{11}C leucine PET/MRI for imaging tumour protein synthesis in colorectal cancer
- P 14** Dr Rico Chi Hang Man
Development of an in vivo traceable triple-negative breast cancer model to study the radiation-induced abscopal effect
- 12:57 Dr Kavitha Sunassee and Dr Gaurav Malviya
An introduction to the UK Preclinical Imaging Network

13:00 **Lunch**

Poster presentations and networking

SESSION 4 TRANSLATION AND THERAPY

Chair *Prof Steve Archibald*

14:30 **O 08** Prof Jane Sosabowski
Preclinical nuclear imaging for radionuclide therapy applications

15:00 **O 09** Ms Philippa Garbutt
Longitudinal ^{99m}Tc-DPD SPECT/CT enables early detection and quantification of disease progression in a mouse model of transthyretin amyloid cardiomyopathy

15:10 **O 10** Prof Hannah F. Clarke
Marmoset dorsolateral prefrontal perineuronal net loss increases caudate dopamine synthesis – relevance to schizophrenia

15:20 **O 11** Mr Nathan Whitty
Multimodal imaging reveals spatial and temporal associations between neuroinflammation, metabolism, and synaptic pathology in experimental multiple sclerosis

15:30 **Coffee break**

SESSION 5

Chair *Dr Nisha Kuzhupilly Ramakrishnan*

15:50 **O 12** Prof Peter Scott
Developing brain imaging agents: from early trial and error to a future of AI-driven CNS PET tracer design

16:50 Prizes and meeting close

17:00 **Drinks reception**

O 01

Advancing Chelator Design to Enhance Preclinical Imaging for Metal-Based Radiotracers

TW Price, L Wagner, RW Kornmann, R Lothe, N Lertthundorn, V Rosecker, I Hawala, and **GJ Stasiuk***

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Positron emission tomography (PET) is a powerful diagnostic tool in nuclear medicine. Typically, a positron emitting radiometal is labelled with a ligand in the (sub)micromolar range within minutes in high chemical and radiochemical yield. Gallium 68 ($t_{1/2} = 68$ min) is the most widely used radiometal for PET due to its good availability and short half-life. Macrocyclic ligands are currently used in clinic but have the disadvantage of slow reaction kinetics. Therefore, there is an interest in developing acyclic ligands with more favourable reaction kinetics. We present a series of acyclic ligands for gallium-68, dpaa [1], tpa [2], bispidine [3] and Bn₂DT₃A [4]. Dpaa and tpa radiolabel efficiently with gallium-68, at pH 7.4 but do not form stable complexes. Bispidine shows high stability but only radiolabelling at pH 4. Bn₂DT₃A gives good radiochemical yields, but different products depending on the pH. At pH 4 [⁶⁸Ga][Ga(Bn₂DT₃A)] is formed and is unstable. At pH 7.4 [⁶⁸Ga][Ga(Bn₂DT₃A)(OH)]⁻ is formed and is stable in serum. *In vivo* studies show rapid renal clearance and negligible unspecific uptake (**Figure 1**). Bn₂DT₃A has been modified to give 1-N₂DT₃A and 2-N₂DT₃A, incorporating naphthyl groups to evaluate the increase in steric hindrance on radiolabelling and stability, giving rise to stable 68Ga complexes at labelled at both pH 4 and 7 with different clearance pathways *in vivo*. Tuning the side arms of X₂DT₃A tunes the PK and BioD of the tracer. X₂DT₃A can be targeted and has been modified with PSMA, [⁶⁸Ga][Ga(X₂DT₃A-PSMA)] and shows targeted imaging of prostate cancer.

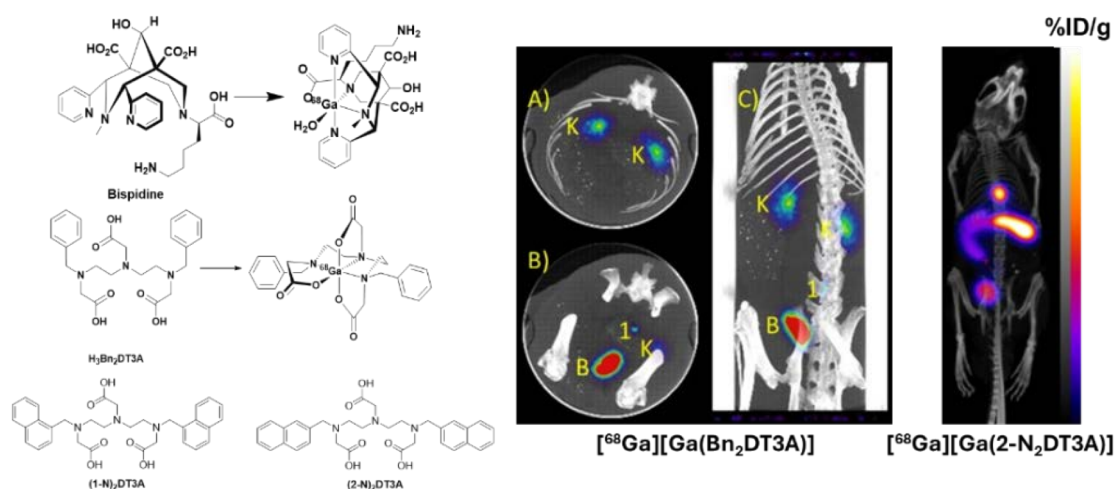


Figure 1. Ga.bispidine, Ga.Bn₂DT₃A, 2N₂DT₃A, 1N₂DT₃A and PET images of [⁶⁸Ga][Ga(Bn₂DT₃A)(OH)]⁻ and [⁶⁸Ga][Ga(2-N₂DT₃A)(OH)]⁻

- [1] Price TW *et al.* Amino acid based gallium-68 chelators capable of radiolabeling at neutral pH. Dalton Trans 2017, 46: 16973–16982
- [2] Price TW *et al.* Inorganic Chemistry of the Tripodal Picolinate Ligand Tpa³⁻ with Gallium(III) and Radiolabeling with Gallium-68. Inorg Chem 2023, 62 (50): 20769–20776
- [3] Price TW *et al.* Evaluation of a Bispidine-Based Chelator for Gallium-68 and of the Porphyrin Conjugate as PET/PDT Theranostic Agent. Chemistry 2020, 26: 7602–7608
- [4] Price TW *et al.* Bn₂DT₃A, a Chelator for ⁶⁸Ga Positron Emission Tomography: Hydroxide Coordination Increases Biological Stability of [⁶⁸Ga][Ga(Bn₂DT₃A)(OH)]⁻

Professor Graeme Stasiuk – Biography



Dr Stasiuk is a Professor in Imaging Chemistry at King's College London. Dr Stasiuk's research includes design and synthesis of novel multimodal imaging agents for MRI, PET and Optical Imaging. These include both small molecules and nanomaterials, based on inorganic complexes and semiconducting nanoparticles/quantum dots. The research is focused on tools for image guided surgery and the development of "smart" theranostic imaging agents capable of monitoring drug delivery/efficacy, in cancer and cardiovascular disease.

O 02

¹⁸F-Radiopharmaceutical Diversification Enabled by Deaminative Cross-Electrophile Couplings

I Ogilvy^{1,*}, J Ford¹, S Ortalli¹, E Renders², T Hayes², S Liu², I Mortiers², A Nikolopoulou², A Sorlin², A Trabanco², M Tredwell³, P Buijnsters², R Salter² and V Gouverneur¹

¹Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford, UK; ²Global Discovery Chemistry, Johnson & Johnson (various sites); ³School of Chemistry, Park Place, Cardiff, UK and Wales Research and Diagnostic PET Imaging Centre, Cardiff University, University Hospital of Wales, Cardiff, UK

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The development of fluorine-18 radiotracers is central to clinical positron emission tomography (PET) imaging and supporting drug discovery campaigns. However, the preparation of complex radiolabelling precursors often limits the rapid generation and evaluation of structural analogues, restricting exploration of radiochemical space. This is highly prevalent in the syntheses of specialized precursors amenable to site-selective, late-stage radio fluorination with weakly nucleophilic [¹⁸F]fluoride (¹⁸F; *t*_{1/2} = 109.7 mins; 97% β⁺ decay; 635 keV) [1]. In this work, we present a nickel-mediated aryl (C)sp²–(C)sp³ cross-coupling strategy compatible with radiosynthesis, employing readily-accessible amine-derived 2,4,6-triphenylpyridinium salts as alkyl coupling partners [2].

In analogy to pharmaceutical discovery, this approach enables late-stage diversification from common precursors, reducing synthetic burden and facilitating access to structurally diverse ¹⁸F-radiotracers. The method is applicable to both primary and secondary *N*-alkyl pyridinium salts, achieving high radiochemical conversions (up to 86%) (**Figure 1**).

A tailored non-radiochemical high-throughput experimentation (HTE) assay was developed to rapidly identify optimal Ni-ligands for structurally diverse fluorinated (hetero)aryl iodides, expediting reaction optimisation. Crucially, the assay results translated well to analogous radiochemical systems. A diversification case study targeting GSK-3 kinase inhibitors demonstrates the utility of this strategy, enabling the synthesis of six ¹⁸F-labelled analogues from a single precursor, including production at gigabecquerel (GBq) scale across three commercial synthesis platforms. This work highlights a novel approach for accelerating radiotracer development through modular and scalable late-stage functionalisation.

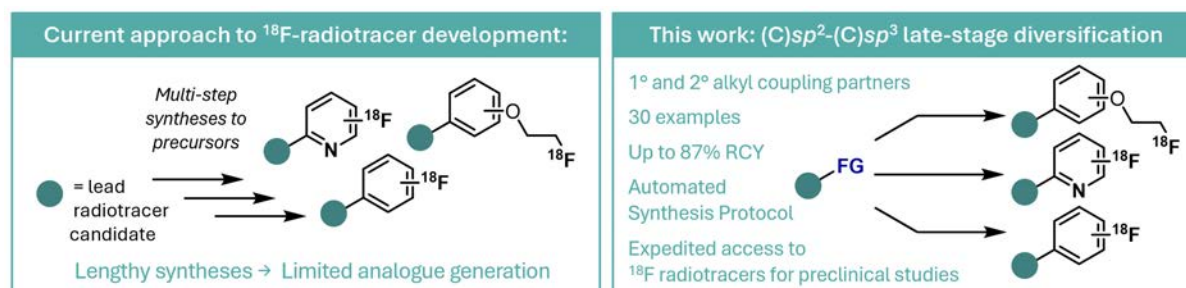


Figure 1. Comparison of multi-step precursor synthesis and late-stage diversification

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- [1] Jacobson O, Kiesewetter DO, Chen X. Harper, M. E. et al. Fluorine-18 radiochemistry, labeling strategies and synthetic routes. *Bioconjugate Chem* 2015, 26, 1: 1–18
- [2] Ogilvy I *et al.* ^{18}F -Radiopharmaceutical Diversification Enabled by Deaminative Cross-Electrophile Couplings. *Angew Chem Int Ed* 2026, 65: e22650

O 03

Development of Reporter Gene-afforded Hypoxia Fate Mapping in Fully Immunocompetent Cancer Models

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Small-animal SPECT/CT with multipinhole collimators offers significant potential for quantitative preclinical imaging. Building on phantom-based characterisation of a novel multipinhole SPECT/CT system, we evaluated its *in vivo* quantitative imaging performance in mice. Five mice were injected with 20 MBq ^{99m}Tc -pertechnetate. Four were imaged across three collimators – APT62 (mouse whole-body), APT66 (mouse focused high-sensitivity) and APT63 (rat/multi-mouse) – with two also undergoing a dynamic 3D neck acquisition over the first 10 minutes post-injection to derive thyroid and salivary gland time-activity curves (TACs), followed by a whole-body SPECT/CT at 50 minutes post-injection. All five mice were culled at 90-110 minutes post-injection and tissues harvested for *ex vivo* gamma counting. Image-derived percent injected activity (%IA) and *ex vivo* tissue uptake was compared against literature biodistribution values at equivalent time points. List-mode SPECT data were retrospectively downsampled to 50% and 10% of acquired counts to assess the impact of count statistics on image quality, TAC fitting, and quantitative accuracy.

Three findings emerged. First, effective resolution varied markedly across collimators: the thyroid was clearly delineated with APT62 (**Figure 1**) and APT66, whereas in mice imaged with collimator APT63, the thyroid could not reliably be differentiated from surrounding tissue. Second, image-derived thyroid quantification was robust to count reduction. Downsampling to 10% of acquired counts produced only a small change in TAC-derived activity at 90 minutes post-injection (relative difference between 100% and 10%: 1.53% and 3.67% for APT62 and APT66 respectively). Third, image-derived thyroid activity at 90 minutes showed a mean paired difference of -0.046 ± 0.344 %IA with gamma counter measurements, confirming quantitative accuracy *in vivo* [1].

These findings demonstrate collimator choice critically determines the effective resolution of small structures: APT63, while enabling higher-throughput multi-mouse imaging, was insufficient to resolve mouse thyroids. The robustness of quantification at 10% of acquired counts suggests scan duration or injected activity can be substantially reduced where adequate contrast and a reference VOI are available, indicating the potential for more flexible or dose-efficient preclinical SPECT study design.

- [1] Franken PR *et al.* Distribution and dynamics of ^{99m}Tc -pertechnetate uptake in the thyroid and other organs assessed by single-photon emission computed tomography in living mice. *Thyroid* 2010, 20 (5): 519–526

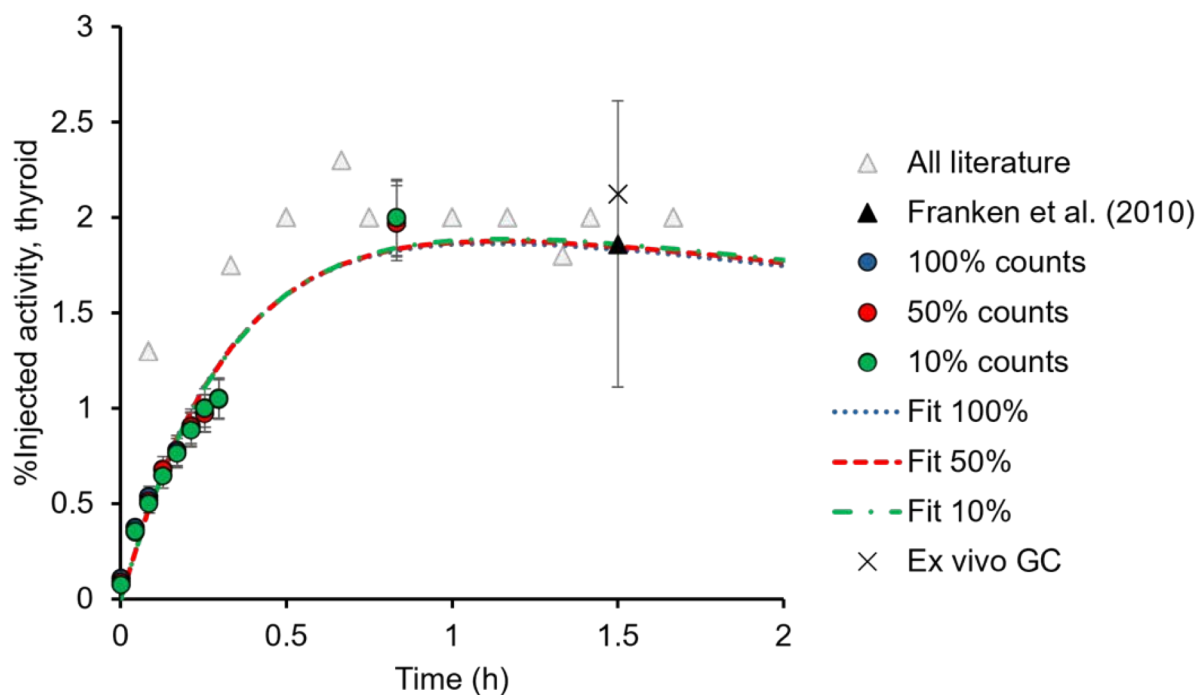


Figure 1. SPECT (APT62)-derived activity at three count levels (100%, 50%, 10%) *versus* *ex vivo* gamma counter (GC) measurement

O 04

Bicycle Radioconjugates: A New Modality for Molecular Imaging of Novel Targets

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The expanding landscape of precision oncology demands novel imaging agents capable of providing rapid, high-contrast visualisation of clinically relevant targets. While antibodies and small molecules can face pharmacokinetic or selectivity trade-offs, Bicycle® peptides bridge this gap. Their compact architecture enables rapid tissue penetration and renal elimination, while their structured binding surface provides antibody-like target affinity and selectivity. Bicycle Radionuclide Conjugates (BRCs) leverage these properties to deliver radioisotopes for diagnostic imaging and radioligand therapy. Here, we demonstrate the robustness of the Bicycle® platform in generating high-contrast, tumor-specific radiopharmaceuticals with favourable biodistribution profiles across diverse oncology targets for molecular imaging and theranostic application.

Dr Gemma E Mudd – Biography



Dr Gemma Mudd is a drug discovery leader currently serving as Radioconjugate Portfolio Lead at Bicycle Therapeutics. Over her tenure at Bicycle, Gemma has focused on developing molecules identified on the novel Bicycle® peptide platform into a class of next-generation clinical candidates. She currently oversees preclinical development programs that utilize Bicycle peptides as targeted vehicles for Bicycle Radionuclide Conjugates (BRCs), pushing forward oncology candidates designed for highly localized cancer imaging and radiopharmaceutical therapy.

O 05

Imaging Tumour Antioxidant Capacity with the Novel xCT-Targeted PET Radiotracer (S)-4-(4-¹⁸F-fluorobenzyl)-L-Glutamate [¹⁸F]FSBG

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Cancer treatment often fails due to tumour adaptation to cytotoxic stress. The cystine–glutamate antiporter xCT promotes resistance by elevating glutathione (GSH) synthesis, protecting cancer cells from oxidative stress and ferroptosis [1]. PET imaging with [¹⁸F]FSPG, an xCT substrate, enables detection of this redox adaptation in patients [2]. Challenging clinical production and off-target organ retention, however, limit its utilisation. Here, we developed (S)-4-(4-¹⁸F-fluorobenzyl)-L-glutamate [¹⁸F]FSBG, a next-generation xCT tracer that overcomes these limitations.

[¹⁸F]FSBG was synthesised by copper-mediated radiofluorination of its corresponding boronic ester precursor using a Trasis All-in-One. The stability of the final formulated [¹⁸F]FSBG was assessed up to 5h post-synthesis by radio-HPLC. *In vitro* uptake assays were conducted using human non-small cell lung cancer (NSCLC) cell lines with high (H460) or low (H1299) xCT expression to assess [¹⁸F]FSBG selectivity. *In vivo*, dynamic [¹⁸F]FSBG and [¹⁸F]FSPG PET imaging was performed in balb/c nu/nu mice bearing subcutaneous H460 or H1299 tumours. *Ex vivo* biodistribution (BioD) at 2h post-injection quantified tracer accumulation in tissues, with IHC analysis used to determine xCT expression.

[¹⁸F]FSBG was synthesised with an isolated radiochemical yield (RCY) of 20-27% over two steps. The final PBS-formulated [¹⁸F]FSBG was stable, with radiochemical purity >98% at 5h post synthesis (**Figure 1A**). In NSCLC cells, [¹⁸F]FSPG and [¹⁸F]FSBG had higher retention in xCT-high H460 cells compared to xCT-low H1299 cells. While both tracers exhibited similar retention in H460 cells, [¹⁸F]FSBG uptake in H1299 cells was lower than that of [¹⁸F]FSPG, with [¹⁸F]FSBG better able to discriminate cells with different xCT levels (**Figure 1B**). PET imaging and *ex vivo* BioD studies in tumour-bearing mice confirmed that [¹⁸F]FSBG accumulation was comparable to [¹⁸F]FSPG in xCT-high H460 tumours, but lower in xCT-low H1299 tumours (**Figures 1C and 1D**). Importantly, [¹⁸F]FSBG showed significantly reduced accumulation in healthy tissues, particularly in the pancreas, compared to [¹⁸F]FSPG (**Figures 1C and 1E**). [¹⁸F]FSBG is a novel xCT-targeting PET radiotracer with sustained tumour retention, improved image contrast over [¹⁸F]FSPG, and a UV-active [¹⁸F]fluorobenzyl group that serves as chromophore for QC, streamlining the clinical use.

[1] Hecht F *et al.* Regulation of antioxidants in cancer. *Molecular Cell* 2024, 84 (1): 23–33

[2] Sharkey AR, Witney TH, Cook GJR. Is System xc- a Suitable Target for Tumour Detection and Response Assessment with Imaging? *Cancers (Basel)* 2023, 15 (23): 5573

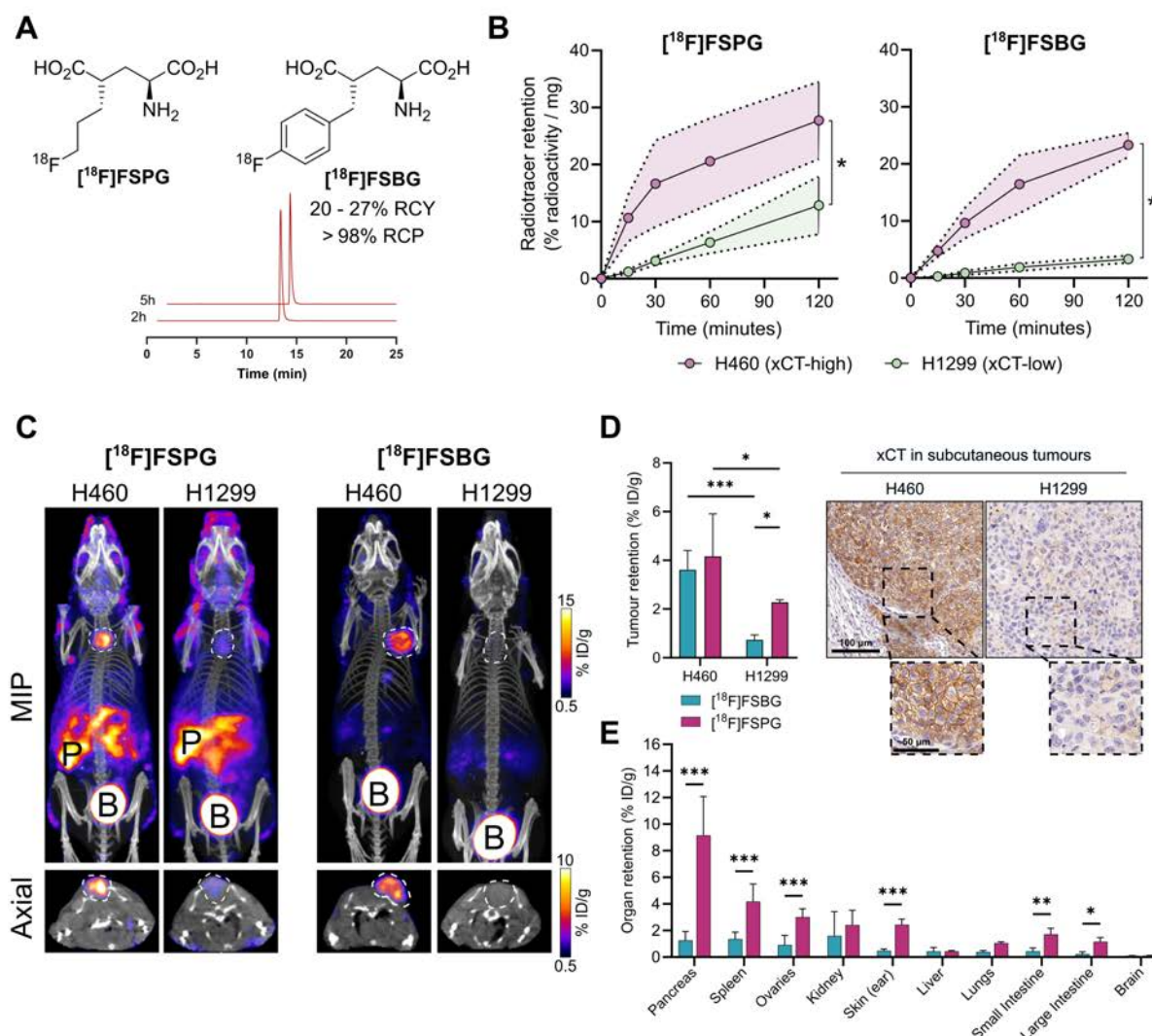


Figure 1. $[^{18}\text{F}]\text{FSBG}$ is a new xCT-targeting radiotracer for PET imaging in cancer. (A) Chemical structures of $[^{18}\text{F}]\text{FSPG}$ and $[^{18}\text{F}]\text{FSBG}$, with representative radio-HPLC chromatogram showing the stability of formulated $[^{18}\text{F}]\text{FSBG}$ in PBS at 2 h and 5 h post synthesis. RCY, radiochemical yield; RCP, radiochemical purity. (B) Uptake and retention of $[^{18}\text{F}]\text{FSPG}$ (left) and $[^{18}\text{F}]\text{FSBG}$ (right) over time in xCT-high H460 or xCT-low H1299 lung cancer cell lines. Statistics were performed using the AUC values for each cell line (unpaired t test, $n = 3$). (C) Representative PET/CT maximum intensity projections (MIPs; top) and axial single-slice PET/CT (bottom) of mice bearing H460 or H1299 subcutaneous tumors injected either with $[^{18}\text{F}]\text{FSPG}$ (left) or $[^{18}\text{F}]\text{FSBG}$ (right) at 100-120 minutes post-injection. Tumors are outlined with dashed lines. P, pancreas. B, bladder. (D) *Ex-vivo* Biod showing tumor retention of $[^{18}\text{F}]\text{FSBG}$ and $[^{18}\text{F}]\text{FSPG}$ in H460 ($n=5$ and $n=4$, respectively) and H1299 ($n=6$ and $n=3$, respectively) xenografts, with corresponding immunohistochemistry images showing xCT expression in the tumors. (E) *Ex-vivo* Biod showing retention of $[^{18}\text{F}]\text{FSBG}$ ($n=11$) and $[^{18}\text{F}]\text{FSPG}$ ($n=7$) in healthy tissues. Bars graphs are presented as mean $\pm$ SD. Uncorrected Fisher's LSD test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

O 06

Polysarcosine Star Dendrimers: An Efficient Drug Delivery System to Tumours with Minimal Accelerated Blood Clearance

T Gibson^{1,2,*}, A Carrascal Miniño², T Floyd³, A Mishra², J Pellico², A Emanuelli², D Jasim¹, K Sunassee², J Sosabowski¹, M Mazza⁴, TH Witney² and RTM de Rosales²

¹Centre for Biomarkers & Biotherapeutics, Barts Cancer Institute, Queen Mary University of London, London, UK; ²Research Department of Imaging Chemistry & Biology, School of Biomedical Engineering & Imaging Sciences, King's College London, London, UK; ³Advanced Drug Delivery, BioPharmaceuticals R&D, AstraZeneca, Macclesfield, UK; ⁴Advanced Drug Delivery, BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK

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Polyethylene glycol (PEG) is the gold standard stealth polymer in nanomedicine. However, concern around anti-PEG immunity, and the resultant accelerated blood clearance (ABC) of PEGylated nanoparticles upon repeat administration, has sparked the search for less immunogenic alternatives, (e.g. polysarcosine (pSar)) [1,2]. We previously established pSar-functionalised dendrimers (SDends) to be long-circulating drug-delivery systems (DDSs) in healthy mice [3,4]. Here, using PET/CT, we determine the impact of pSar functionalisation on the tumour uptake of SDends following one-off or repeat administration in an immunocompetent 4T1 mouse model.

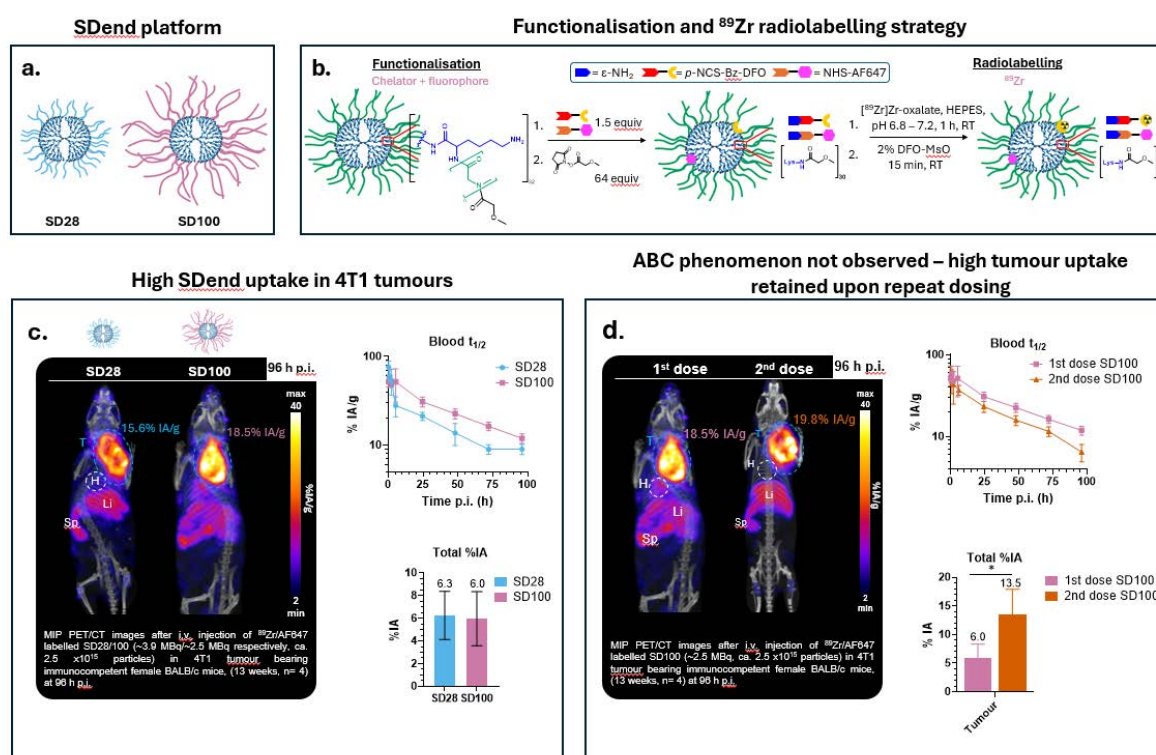
Two SDends (SD28/SD100) were synthesised, functionalised with deferoxamine (p-NCS-Bz-DFO), Alexafluor647 (AF647-NHS) and radiolabelled with neutralised [⁸⁹Zr]Zr-oxalate (**Figures 1a and 1b**). To study differences in biodistribution, pharmacokinetics and tumour uptake, [⁸⁹Zr]ZrSD28/100-DFO-AF647 were administered intravenously to immunocompetent 4T1 tumour-bearing female BALB/c mice (3 MBq, 2.5 x 10¹⁵ particles) and PET/CT images acquired 1.5, 24, 48, 72 and 96 h post-injection (p.i.). To study the impact of repeat dosing, mice were first administered unfunctionalised SD100 on Day 0, followed by [⁸⁹Zr]ZrSD100-DFO-AF647 on Day 6.

SDends retained long circulation times in this disease model ($t_{1/2}$ = 31.1±2.3 and 46.6±2.7 h, SD28 and SD100 respectively, **Figure 1c**). High uptake was observed in 4T1 tumours, achieving 15.6±2.7 and 18.5±1.6% IA/g, or 6.3±2.1 and 6.0±2.4% IA 96 h p.i. for SD28 and SD100 respectively. This is remarkably higher than the reported median uptake (0.7% ID) of nanoparticles into tumours [5], highlighting SDends ability to evade immune scavenging and exploit passive tumour accumulation mechanisms. Furthermore, the ABC phenomenon was not apparent, with the biodistribution of SD100 remaining largely unaffected by repeat administration, achieving equivalent tumour (19.8±2.6% IA/g) and liver uptake (10.3±1.6% IA/g) to naïve mice (**Figure 1d**). While blood $t_{1/2}$ decreased upon repeat administration (30.5±8.7 h), this is attributed to the greater overall tumour uptake (13.5% IA) given the larger tumour mass.

We reveal the stealth capability of pSar is retained in a tumour model, with SDends facilitating efficient passive tumour targeting. SDends also do not trigger the ABC phenomenon, further

demonstrating the non-immunogenic nature of pSar. This work demonstrates the potential for SDends as a novel drug delivery platform with high therapeutic potential.

- [1] Chen BM, Cheng TL, Roffler SR. Polyethylene Glycol Immunogenicity: Theoretical, Clinical, and Practical Aspects of Anti-Polyethylene Glycol Antibodies. *ACS Nano* 2021, 15: 14022–14048
- [2] Son K *et al.* Evasion of the accelerated blood clearance phenomenon by polysarcosine coating of liposomes. *Journal of Controlled Release* 2020, 322: 209–216
- [3] England RM *et al.* Synthesis and Characterization of Dendrimer-Based Polysarcosine Star Polymers: Well-Defined, Versatile Platforms Designed for Drug-Delivery Applications. *Biomacromolecules* 2020, 21: 3332–3341
- [4] Gibson T *et al.* Multimodal PET/Fluorescence Imaging for the Preclinical Evaluation of Polysarcosine (pSar)-modified Dendrimers: Impact of pSar Length on in vivo fate. *ChemRxiv* 2026, <https://doi.org/10.26434/CHEMRXIV.15008001/V1>
- [5] Wilhelm S *et al.* Analysis of nanoparticle delivery to tumours. *Nature Reviews Materials* 2016: 16014.



O 07

²²⁵Ac/⁸⁹Zr-hNd2: Novel Theranostic Agents Targeting MUC5AC-Positive Cancers

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Mucin 5AC (MUC5AC) is a secreted mucin, with aberrant overexpression in multiple malignancies including pancreatic, gastrointestinal, and liver cancers. Pancreatic cancer in particular has a dismal 5-year survival rate. Therapeutic progress has been hampered by major challenges to drug development, such as a dense desmoplastic stroma, limiting drug delivery, and tumour hypoxia, which contributes to therapeutic resistance. To address these unmet needs, this study aimed to develop a novel antibody-based theranostic agent specifically targeting MUC5AC-positive tumours, including pancreatic cancer. This approach leverages the potent cytotoxic properties of ²²⁵Ac, enabling targeting of cancer cells from the extracellular compartment even under hypoxic conditions. Antibody modification was performed using the Chemical Conjugation by Affinity Peptide (CCAP) method, which preserves antigen binding properties while facilitating antibody clearance.

A humanized anti-MUC5AC antibody (hNd2) was site-specifically modified at the Fc region using the CCAP method and subsequently radiolabelled with ⁸⁹Zr (diagnostic agent, NMK89) and ²²⁵Ac (therapeutic agent, NMT25) [1]. Binding affinity was determined by saturation binding assays using recombinant MUC5AC protein, specificity was assessed by in vitro autoradiography with human pancreatic cancer tissue sections. Image-based biodistribution studies were performed in marmosets, and tumour uptake was evaluated by PET imaging in tumour-bearing mice. NMT25 therapeutic efficacy was investigated in MUC5AC-positive subcutaneous xenograft models.

In saturation binding assays, hNd2 demonstrated high binding affinity for MUC5AC (K_D=2.3 nM). In vitro autoradiography showed specific accumulation of NMK89 in MUC5AC-positive tumour lesions, which were confirmed by immunohistochemistry. In marmoset biodistribution studies, PET imaging was performed up to 168 hours post-administration, and NMK89 exhibited rapid clearance from all organs and tissues without unexpected accumulation. PET imaging across multiple xenograft mouse models revealed high tumour uptake with sustained retention and favourable clearance kinetics in normal organs and tissues. In efficacy studies in mice, NMT25 induced prolonged, dose-dependent inhibition of tumour growth following a single administration (**Figure 1**).

Preclinical studies demonstrate that NMK89 and NMT25 are promising theranostic agents for MUC5AC-positive cancers, showing clear tumour targeting and potent antitumor efficacy in experimental models. Tumour accumulation of NMK89 has been confirmed in human pancreatic cancer patients (NCT06129422), and clinical evaluation of NMT25 is planned.

- [1] Nakata N *et al.* Radiation dosimetry and efficacy of an $^{89}\text{Zr}/^{225}\text{Ac}$ -labeled humanized anti-MUC5AC antibody. *Nucl Med Biol* 2022, 108–109: 33–43

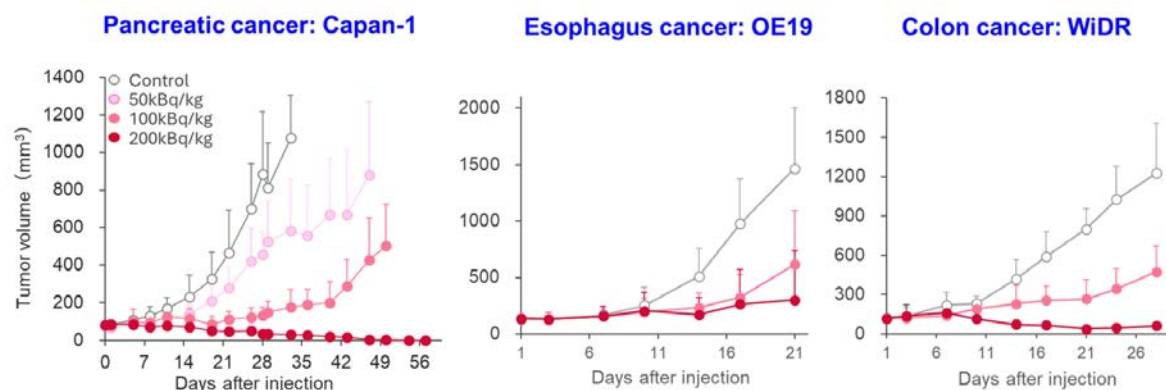


Figure 1. NMT25 induced prolonged, dose-dependent inhibition of tumour growth

O 08

Preclinical Nuclear Imaging for Radionuclide Therapy Applications

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With new therapy radionuclides (including alpha particle emitters) becoming more widely available for preclinical research, questions remain about how best to pair these with imaging radionuclides when translating to the clinic for theranostic use. Although the ideal is to pair with a suitable radioisotope of the same element, in some cases this is not possible or practical. Decay chains involving multiple radioactive daughters can also introduce differences in biodistribution for radionuclides of the same element. Data will be presented on biomolecules radiolabelled with isotopes of lead and other radionuclides, including *in vivo* imaging-based assessment of toxicity and dosimetry.

Prof Jane Sosabowski – Biography



Professor Jane Sosabowski is group leader at the Barts Cancer Institute at Queen Mary University of London. Her research focuses on the development of radiopharmaceuticals for imaging and therapy of cancer, and translating these to the clinic. She is particularly interested in the use of alpha particle emitters for targeted radionuclide therapy. Her group is developing methods for non-invasive imaging and quantitation of genetically modified cell therapies *in vivo* to provide data on their biodistribution, expansion and efficacy over time. They have also developed and implemented computational machine learning tools for defining disease burden in models of pancreatic cancer using MRI imaging to reduce the numbers of animals used in scientific research.

Jane graduated with a BSc (Hons) in Chemistry from University of Natal, Durban, South Africa and went on to carry out an MSc by research into the Photochemistry of Sunscreen Constituents at the same university. She then moved to the UK to the Joint Department of Physics at the Institute of Cancer Research in Sutton, Surrey and completed a PhD looking at the development of PET radiotracers for the *in vivo* assessment of multidrug resistance. She then joined the Nuclear Medicine Research Laboratory at Barts and the London School of Medicine and Dentistry as a post doc with Prof Stephen Mather, before becoming a group leader at the same institution.

O 09

Longitudinal ^{99m}Tc -DPD SPECT/CT Enables Early Detection and Quantification of Disease Progression in a Mouse Model of Transthyretin Amyloid Cardiomyopathy

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Transthyretin amyloid cardiomyopathy (ATTR-CM) is characterised by progressive myocardial deposition of misfolded transthyretin (TTR). Bone-avid radiotracers, including ^{99m}Tc -DPD, have transformed clinical detection by enabling non-invasive diagnosis [1], however, their relationship with progressive amyloid burden and underlying disease biology remains incompletely understood. Here, we longitudinally evaluated ^{99m}Tc -DPD SPECT/CT alongside complementary imaging and tissue proteomics in a recently established mouse model of ATTR-CM.

Transgenic mice expressing human amyloidogenic TTRS52P underwent intravenous ATTR fibril seeding to initiate amyloid deposition [2]. At baseline and 4, 8 and 12 months post-seeding, ATTR and unseeded control cohorts (n=8/group) underwent ^{99m}Tc -DPD SPECT/CT, cardiac MRI, ultrasound and microCT. Cardiac ^{99m}Tc -DPD uptake was quantified using SUV_{max} and compared with MRI-derived extracellular volume (ECV) and cardiac function. Cardiac tissue underwent label-free quantitative proteomics, Congo red staining and ATTR immunohistochemistry.

^{99m}Tc -DPD SPECT/CT detected myocardial amyloid involvement from 4 months post-seeding, with cardiac SUV_{max} increased in ATTR compared with control mice (1.33 ± 0.73 vs 0.25 ± 0.03), rising to 2.41 ± 0.78 at 8 months before plateauing at 2.42 ± 0.45 at 12 months. Regional analysis demonstrated increasingly heterogeneous myocardial uptake, with significant apical-to-basal differences emerging at 8 and 12 months (ANOVA $p=0.029$ and $p=0.005$, respectively). A marked phenotypic transition occurred between 4 and 8 months, with increasing LVM and ECV and declining longitudinal function (MAPSE). Proteomic changes progressed in parallel, with early accumulation of TTR and canonical amyloid-associated proteins, including APOE, APOA4 and vitronectin. Pathway analysis demonstrated early extracellular matrix remodelling and haemostatic/platelet processes, followed at 8 months by complement and immune activation alongside suppression of mitochondrial energy metabolism. These molecular alterations persisted and evolved at 12 months despite plateauing myocardial ^{99m}Tc -DPD uptake. Congo red staining and ATTR immunohistochemistry confirmed myocardial ATTR deposition.

Longitudinal ^{99m}Tc -DPD SPECT/CT detected early cardiac amyloid involvement, preceding marked structural and functional abnormalities. A critical 4-8-month transition was characterised by concurrent imaging and molecular changes, with continued proteomic remodelling despite later stabilisation of ^{99m}Tc -DPD uptake. This model provides a translational platform for evaluating ATTR disease progression and emerging therapeutics.

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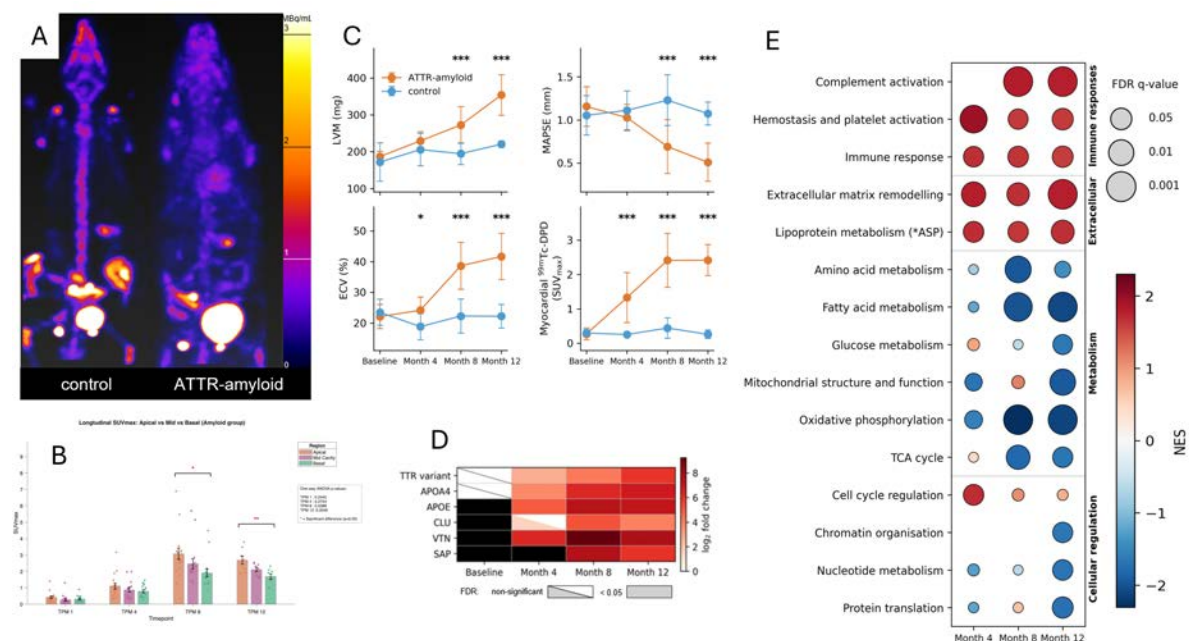


Figure 1. Longitudinal imaging and proteomic changes in the ATTR-amyloid mouse model during disease progression. (A) Multimodal imaging measurements including myocardial ^{99m}Tc -DPD uptake (SUV_{max}), MRI-derived extracellular volume (ECV), left ventricular mass (LVM), and mitral annular plane systolic excursion (MAPSE) in ATTR-amyloid and control mice (mean $\pm$ SD). $P < 0.05$ (*), $P < 0.001$ (***). (B) Regional SPECT analysis demonstrates the emergence of heterogeneous myocardial ^{99m}Tc -DPD uptake. (C) Heatmap of representative amyloid-associated proteins. Black cells indicate proteins not detected; colour intensity represents log₂ fold change in ATTR-amyloid relative to controls, and fully coloured cells indicate significant differential expression (FDR < 0.05). (D) Representative ^{99m}Tc -DPD SPECT/CT scans of control and ATTR-amyloid mice at 12 months post-seeding. (E) Pathway enrichment of differentially expressed proteins grouped into broader biological processes. Circle colour represents normalized enrichment score (NES) for ATTR-amyloid relative to controls, and circle size represents FDR q-value. *ASP denotes Amyloid Signature Proteins identified within the enriched pathways.

O 10

Marmoset Dorsolateral Prefrontal Perineuronal Net Loss Increases Caudate Dopamine Synthesis – Relevance to Schizophrenia

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²Molecular Imaging Chemistry Laboratory, Department of Clinical Neurosciences, University of Cambridge; Cambridge Biomedical Campus, West Forvie building, Robinson Way, CB2 0SZ, Cambridge, UK; ³Wolfson Brain Imaging Centre, University of Cambridge; Cambridge UK

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Schizophrenia is a severe, incurable psychiatric disorder that is associated with dysfunction in both the prefrontal cortex (PFC) and the striatum whose origins are unknown. Neuroimaging research suggests that dysfunction within the dorsolateral PFC (dlPFC) may contribute to the increased striatal dopamine synthesis observed in patients with schizophrenia [1]. However, the causal role of the dlPFC and dlPFC pathology in regulating striatal dopamine is unclear. We hypothesised that recreating aspects of dlPFC pathology that present in schizophrenia postmortem would reveal their contribution to striatal dopamine function. Therefore, we used a species with a human-like prefrontal cortex, the marmoset monkey, to investigate one aspect of this pathology, the loss of the dlPFC extracellular matrix structure, the perineuronal nets [2].

We cannulated 4 marmosets (*Callithrix jacchus*) in the dlPFC (areas 9 and 46). This allowed the infusion of Chondroitinase ABC – an enzyme that selectively degrades the PNN. We then used ¹⁸F-DOPA PET with MRI template co-registration to quantify dopamine synthesis throughout the subregions of the striatum both before, and after dlPFC perineuronal net degradation. When the measures of dopamine synthesis in the striatum taken before dlPFC perineuronal net degradation were compared to those taken after dlPFC perineuronal net degradation regionally specific alterations were seen. Specifically, dopamine synthesis was increased within the caudate nucleus only, and no changes were seen in the putamen, nucleus accumbens, globus pallidus or other subregions of the caudate nucleus. Histology confirmed that PNN degradation was limited to areas 9/46D.

These data provide causal evidence that recapitulation of dlPFC pathology that is observed in schizophrenia is sufficient to increase striatal dopamine synthesis. Crucially the location of this striatal increase matches the pattern of the usually observed in clinically [3]. Furthermore, they suggest a crucial role for dlPFC perineuronal nets in the development of schizophrenia

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O 11

Multimodal Imaging Reveals Spatial and Temporal Associations between Neuroinflammation, Metabolism, and Synaptic Pathology in Experimental Multiple Sclerosis

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Multiple Sclerosis (MS) is a chronic neuroinflammatory disease of the central nervous system (CNS), in which demyelination and neurodegeneration contribute to progressive neurological dysfunction. Most patients initially experience relapse-remitting disease (RRMS), driven initially by myelin-targeting autoimmune cell infiltration into the CNS and typically transitioning into progressive disability (pMS) overtime, whilst others present with pMS from disease onset [1,2]. pMS is characterised by persistent ‘smouldering’ inflammation that is maintained by inflammatory glial cells, culminating in compartmentalised neurodegeneration that contributes to cognitive impairment in pMS patients [3–5]. Effective treatments targeting these mechanisms remain limited, highlighting a major unmet need to understand and therapeutically target the neuropathology underlying cognitive decline in MS. Recent evidence has demonstrated microglial metabolic dysfunction as a causative mechanism of neurodegeneration within chronic experimental autoimmune encephalomyelitis (EAE), a mouse model of pMS [6]. Pro-inflammatory microglia experience a metabolic shift towards aerobic glycolysis *in vitro*, diverting pyruvate from mitochondrial oxidative phosphorylation toward cytosolic fermentation into lactate as a means for ATP generation [7]. However, how microglial-mediated local CNS lactate dynamics affects neuron health and function during chronic inflammatory disease is not understood.

Utilizing successive positron emission tomography (PET) modalities targeting the microglial activation marker TSPO and presynaptic vesicle protein SV2A, we demonstrate colocalised microgliosis and synaptopathology within posterior CNS regions in chronic EAE mice, evidencing a potential spatial relationship between compartmentalised microglial inflammation and synapse loss. Hyperpolarised [1-¹³C] pyruvate magnetic resonance spectroscopic imaging (HP-MRSI) indicated raised whole-brain glycolytic flux within the subacute, but not chronic, stage of disease, highlighting temporally distinct metabolic alterations with potential stage-sensitive neurotoxic or neuroprotective consequences. Preliminary TSPO PET in subacute EAE suggested this raised lactate production may be associated with widespread myeloid inflammation, involving both activated microglia and infiltrating myeloid cells, that express high levels of the lactate-producing enzyme LDHA.

Together, these findings demonstrate the feasibility of integrating complementary imaging modalities to interrogate the spatial and temporal relationships between chronic neuroinflammation, altered metabolism, and synaptopathology. This multimodal approach provides a foundation for dissecting dysfunctional neuroimmunometabolic mechanisms underlying disease progression and cognitive impairment in pMS.

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- [4] Möck EEA, Honkonen E and Airas L. Synaptic Loss in Multiple Sclerosis: A Systematic Review of Human Post-mortem Studies. *Front Neurol*. 2021, 12: 782599
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KEYNOTE LECTURE – O 12

Developing Brain Imaging Agents: From Early Trial and Error to a Future of AI-Driven CNS PET Tracer Design

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Brain PET imaging has revolutionized the diagnosis of neuropsychiatric disorders, enabling early and accurate staging of disease, confirming eligibility of patients for new treatments, and monitoring their response to therapy. Given this, PET is now also widely used to support drug discovery efforts in pharma neuroscience programs. However, the process of designing and synthesizing a new brain PET tracer remains laborious and involves significant trial and error, with failures (e.g. for lack of brain uptake or low radiochemical yield) often not evident until considerable time has been spent on precursor synthesis, radiochemistry and/or preclinical imaging studies. Such challenges are becoming even more difficult as nuclear medicine grows, committing more infrastructure and workforce to routine clinical tracer production, and a new paradigm for tracer development is urgently needed. Existing methods to accelerate tracer development and reduce probe failures fall short; scoring methods (e.g. multiparameter optimization, MPO) used to design and filter CNS tracer candidates for BBB permeability are based on therapeutic drugs and often fail for PET tracers, while one-variable-at-a-time (OVAT) approaches to radiosynthesis optimization are inadequate for newer multicomponent radiochemistry reactions. This talk will introduce new AI-driven approaches that utilize both high-throughput experimentation and machine learning to accelerate tracer design and radiosynthesis. Results to date show BBB predictions that outperform existing MPO methods, and completion of radiochemistry optimization in a few days, compared to several months using traditional OVAT approaches.

Professor Peter Scott – Biography



Peter JH Scott is the Paul L. Carson Collegiate Professor of Radiology at the University of Michigan, with courtesy appointments in Medicinal Chemistry and Pharmacology, Director of the PET Center and the Associate Chair for Research. Prof. Scott trained in the UK (Loughborough, Durham), before moving to the US for post-doc (Buffalo, Michigan), and he started his faculty career at the University of Michigan in 2009. Today, Scott's group is involved in all aspects of the Radiopharmaceutical Sciences including: i) developing new methods for producing radionuclides and radiolabelling bioactive molecules, ii) designing and translating new radiotracers for PET imaging, iii) cGMP radiopharmaceutical manufacture, and iv) adapting new technology (e.g. artificial intelligence) to imagine the radiochemistry laboratory of the future. Peter has published about 250 papers and over 30 book

chapters, about 300 conference abstracts and edited 5 books. He is an inventor on multiple patents and has received numerous awards for his research including both the 2023 Sam Gambhir Trailblazer Award and the 2026 Michael J Welch Award from SNMML. His laboratory is funded by the National Institutes of Health, and has multiple collaborations with academic institutions, biotech and pharma companies all over the world.

P 01

Fluorescent Antagomir 223 Probes as a Prototype for microRNA Targeted Nuclear Imaging

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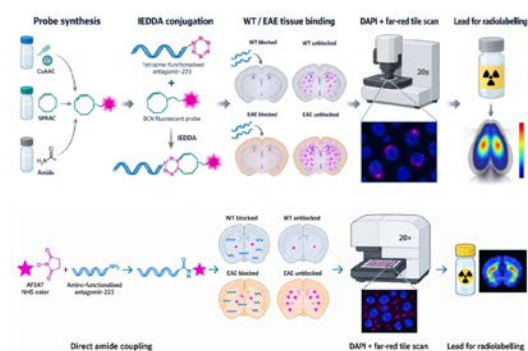
Neuroinflammation is a hallmark of many neurological disorders but remains difficult to characterise non-invasively. MicroRNA-223 (miR-223), a regulator of neuroimmune responses implicated in neuroinflammatory diseases, including multiple sclerosis (MS), represents a promising target for molecular brain imaging. We developed fluorescent anti-miR-223 probes as chemically and biologically traceable prototypes for subsequent radiolabelling.

Fluorescently labelled antagomir probes were prepared using complementary bioconjugation strategies. Three fluorescent tags were prepared using click chemistry (copper-catalysed azide–alkyne cycloaddition (CuAAC), strain-promoted azide–alkyne cycloaddition (SPAAC)), or directly with amide coupling. The resulting bicyclononyne-functionalised fluorescent probes were subsequently conjugated to tetrazine-functionalised antagomir-223 by inverse-electron-demand Diels–Alder (IEDDA) ligation, yielding fluorescent antagomir-223 conjugates, which were analysed through High-Pressure Liquid Chromatography. An ex-vivo fluorescence imaging protocol was then developed by adapting principles from autoradiography and immunohistochemistry workflows. The fluorescent antagomir probes were applied to experimental autoimmune encephalomyelitis (EAE; a model of multiple sclerosis) and wild-type mouse brain sections, alongside matched blocked sections containing excess unconjugated antagomir. DAPI and far-red fluorescence were acquired by 20× whole-slide tile-scanning microscopy (**Figure 1A**).

Three functionalised antagomir probes were obtained on a microgram scale in 15 minutes, under mild biorthogonal conditions compatible with RNA integrity. In preliminary tissue experiments, both click-derived and amide-coupled probes produced nuclear-proximal candidate puncta. Exploratory analysis of these fluorescent candidates showed higher candidate densities in unblocked sections than in their respective matched blocked controls. Among the unblocked sections, candidate densities were higher in EAE than in WT tissue (**Figure 1B**).

The workflow provides a practical fluorescence-stage test of biorthogonal antagomir conjugation and tissue localisation before introducing radiochemical constraints. The preliminary microscopy supports continued optimisation and demonstrates the feasibility of using fluorescent antagomirs to evaluate them as potential targets before radiolabelling. Optimisation and replication with additional animals will guide selection of the lead conjugation strategy for radiolabelling and evaluation as a miR-223-targeted nuclear imaging probe.

A. Graphical Summary



B. miR-223 localization in EAE and WT brain tissue

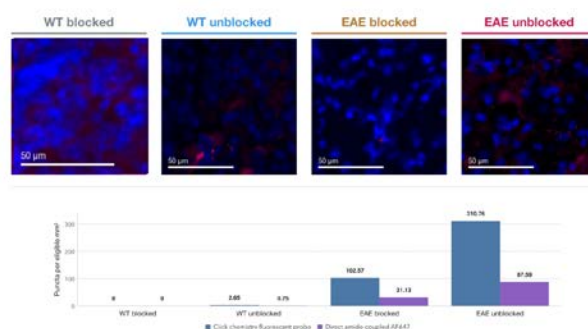


Figure 1. Graphical summary of project workflow (A) and miR-223 staining in mouse brain tissue

P 02

GPR17 Antagonists as PET Radiotracers to Visualise Oligodendrocyte Progenitor Cells in Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system characterised by demyelination, neuroinflammation, and progressive physical and neurological disability. In healthy individuals, oligodendrocyte progenitor cells (OPCs) play a key role in repairing damaged myelin. However, this process is impaired in MS patients. For potential regenerative therapies aiming to restore its function, treatment strategy will differ depending on whether lesions contain OPCs that are failing to differentiate or ones that are OPC-deficient, a distinction that cannot currently be made *in vivo*. This project aims to develop positron emission tomography (PET) radiotracers targeting G-protein coupled receptor 17 (GPR17), a marker of differentiation-committed OPCs, to allow for this distinction to be made and inform more effective treatment options.

Pharmaceutical patents describing GPR17 antagonists were screened for reported receptor-binding data, with compounds reported to have an IC₅₀ of ≤ 10 nM considered as potential leads. Candidates were then ranked for blood–brain barrier (BBB) permeability using six publicly available machine-learning models, with rankings averaged across models to improve accuracy. Lead compounds were selected based on reported binding affinity, predicted BBB penetration, and synthetic feasibility. Synthetic routes to the reference compounds and prospective radiolabelling precursor compounds were designed, and reference compound synthesis was attempted accordingly.

Screening of patents yielded 188 compounds meeting the binding threshold. Ranking these compounds using computational BBB screening revealed that the most BBB penetrant of these compounds mostly originated from a single UCB patent. These compounds have a sulfonamide-based skeleton and predominantly feature a 6-chloroindole motif. Compound I-141 was ranked most BBB-penetrant with I-116 additionally selected as a lead based on strong reported antagonism and reagent availability. I-116 was successfully synthesised via a route combining patent and literature procedures and characterised by NMR and LCMS, representing the first reference compound obtained for this project. Several GPR17 antagonists have been identified as promising PET radiotracer candidates for imaging OPCs in MS. Successful synthesis and characterisation of I-116 provides a foundation for precursor development. Future work will focus on synthesising the designed radiolabelling precursor for I-116, followed by radiolabelling and biological evaluation to assess its suitability for *in vivo* OPC imaging.

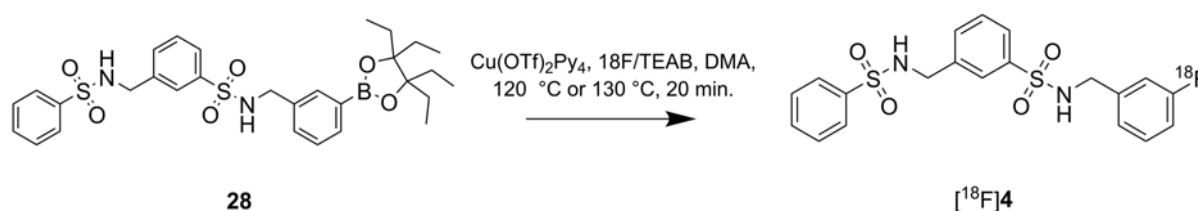
P 03

First Radiosynthesis of a GluA2-Targeted AMPA Receptor PET Radioligand via Copper-Mediated RadiofluorinationNMF Nik Afinde^{1,*}, S Zeng¹, Y Zhao¹, M Zlatar², FI Aigbirhio¹ and SM Sephton¹¹Molecular Imaging Chemistry Laboratory, Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK; ²Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia
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Synaptic loss best tracks cognitive decline in Alzheimer's disease (AD), yet the clinical PET biomarker of synaptic density, SV2A, is purely presynaptic and cannot report postsynaptic AMPA receptor (AMPA) loss, an early event driven by soluble amyloid- β oligomers and mislocalised tau. Existing GluA2-directed PET tracers ($[^{11}\text{C}]\text{K-2}$, $[^{18}\text{F}]\text{K-40}$) undergo rapid in vivo hydrolysis of a metabolically labile amide, requiring arterial blood sampling for kinetic quantification. We report the design, computational selection and first radiosynthesis of our 52E series (52E17), a bis-sulfonamide GluA2 positive-allosteric-modulator (PAM)-site ligand engineered to remove this liability.

A 344-compound library derived from the PEPA scaffold was docked against the GluA2 PAM site (PDB 3M3L) using AutoDock Vina and CCDC GOLD (ChemPLP), cross-validated against 69 known complexes (pose reproduction within 3.0 Å RMSD). Candidates were filtered by predicted blood–brain-barrier permeability (BBB score, CNS-MPO, CNS PET MPO) and ranked against experimental surface plasmon resonance (SPR) affinity. The selected lead from our 52E series ($K_D = 86.7$ nM) carries an arylboronate handle enabling ^{18}F -labelling by copper-mediated radiofluorination (CMRF). Non-automated CMRF was performed manually using $\text{Cu}(\text{OTf})_2(\text{py})_4$ in DMA across a temperature/time screen (120–130 °C, 5–20 min).

Our 52E series lead was the only candidate with a reliably saturating SPR binding curve, despite ranking outside the top three by docking score, showing that affinity ranking required orthogonal experimental validation. A 100 ns molecular dynamics simulation confirmed a stable binding pose (ligand RMSF 2.46 Å) anchored to Ser497 and Ser729 of the GluA2 ligand-binding domain. Radiofluorination of our 52E series gave the highest crude radiochemical conversion (27%) at 130 °C/10 min; extending to 20 min reduced conversion to 12%, consistent with thermal decomposition (**Figure 1**). A dominant unassigned species (77–81% of crude activity, tentatively an *N*-fluorosulfonamide) co-forms under all conditions tested. This is, to our knowledge, the first reported radiosynthesis of an AMPAR GluA2 PAM-site tracer by CMRF of an arylboronate precursor.

**Figure 1.** Radiosynthesis of lead compound

CMRF is a viable route to this metabolically redesigned AMPAR PET tracer, establishing the foundational radiochemistry for our 52E series. Suppressing the competing side product and isolating a BEpin precursor are the immediate priorities ahead of autoradiography and dynamic PET imaging in Wistar rats.

P 04

Development of Second-Generation Difluoromethylated PET Tracers for Huntington Aggregate Imaging

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The development of PET radiotracers for imaging mutant huntingtin (mHTT) fibrils remains an important objective in Huntington's disease research, enabling investigation of disease pathology and potentially supporting therapeutic development. [¹⁸F]difluoromethylation is an attractive strategy in radiotracer design due to its favourable effects on physicochemical and pharmacokinetic properties, and recent development of a practical [¹⁸F]difluorocarbene reagent by Sap and co-workers [1] has significantly expanded access to such tracers. Previous work within our group established an automated [¹⁸F]difluoromethylation methodology [2,3] for a series of huntingtin-targeted ligands derived from structures reported through the CHDI Foundation [4]. While the resulting compounds demonstrated promising characteristics, instability of precursor molecules under radiolabelling conditions resulted in low radiochemical yields, limiting further biological evaluation.

To address these challenges, a second generation of ligands has been designed based on the previously identified binding scaffold. In the new series, the linker connecting key pharmacophoric fragments is systematically varied to investigate its influence on precursor stability during radiolabelling, as well as target binding and brain uptake, while maintaining favourable molecular properties.

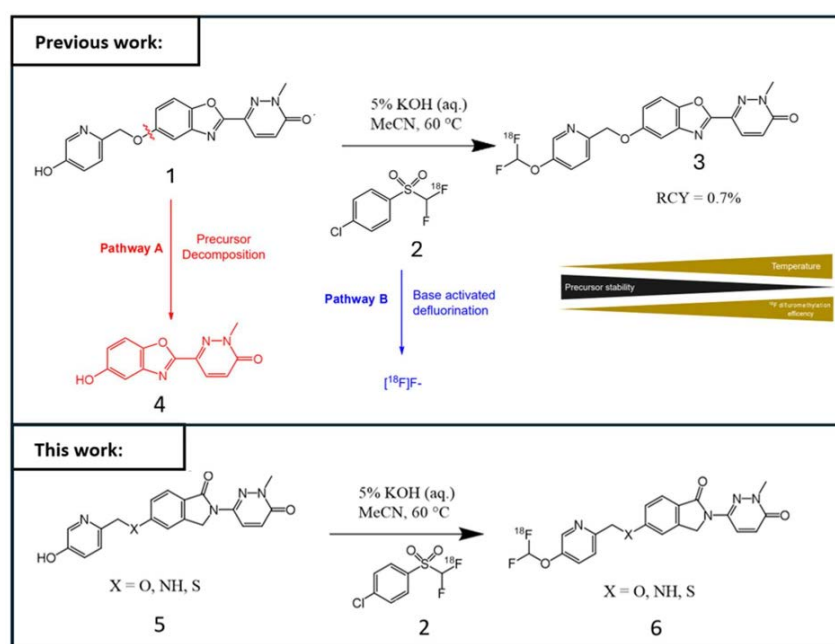


Figure 1. Summary of previous and presented work

A compound library comprising 28 ligand designs was developed to systematically investigate the influence of ether, thioether and amine linkers on the properties of potential mutant huntingtin PET radiotracers. The library was evaluated using CNS MPO scoring, with an isoindoline-based scaffold identified from recent CHDI studies forming the basis of the designed series due to its reported high affinity for mutant huntingtin aggregates. The resulting compounds showed favourable CNS MPO profiles across the different linker classes, supporting their selection for experimental synthesis. A modular synthetic strategy employing common intermediates and Buchwald-type cross-coupling reactions has been developed to facilitate rapid analogue preparation. Initial synthetic work has yielded the first precursor compounds.

Systematic variation of linker structure provides a strategy to investigate its influence on the radiochemical and biological properties of potential mutant huntingtin PET radiotracers. Ongoing work focuses on completing selected precursor compounds and their radiolabelling. Subsequent biological evaluation will investigate linker effects on target binding and brain uptake, with the aim of identifying candidates for future preclinical imaging studies in Huntington's disease.

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P 05

Synthesis of Fluorinated *N*-Acylethanolamine Acid Amidase (NAAA) Inhibitors to Enable the Development of a Radiotracer Targeting NAAA

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N-Acylethanolamine acid amidase (NAAA) is a lysosomal *N*-terminal hydrolase that regulates levels of palmitoylethanolamide (PEA) and plays a key role in inflammatory and pain processes by terminating PEA-mediated anti-inflammatory signalling [1]. Through modulation of PEA signaling, NAAA has been a promising target to understanding the mechanism of neuroinflammation and its role in multiple sclerosis (MS). Moreover, non-invasive imaging of neuroinflammation is important for understanding disease progression and evaluating therapeutic interventions in MS. Positron emission tomography (PET) enables in vivo visualization of molecular targets with the high resolution [2]; however, to the best of our knowledge, no PET radiotracer has yet been reported for imaging NAAA in either peripheral tissues or the central nervous system. To address this gap, we aimed to develop a PET radiotracer targeting NAAA. As a starting point, with the aim of developing both a PET radiotracer and a potential therapeutic, we selected ARN19702, a potent and selective NAAA inhibitor developed by Piomelli and co-workers [3], as the lead structure for tracer design. In this study, a comprehensive structure–activity relationship (SAR) investigation based on fragment-based approach was conducted utilising the crystal structure of NAAA available.

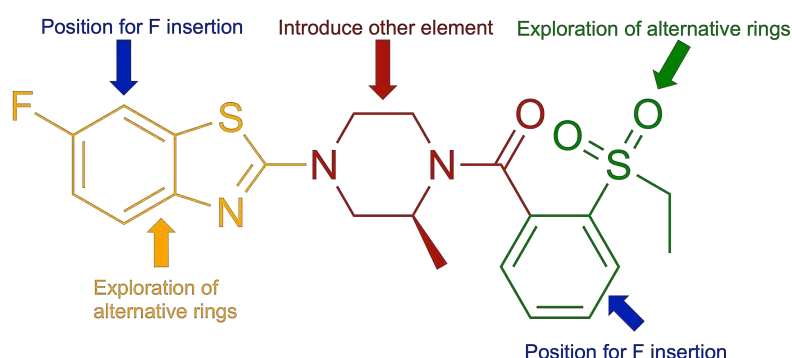


Figure 1. Structure-activity relationships for NAAA inhibitor development

Promising candidates were radiolabeled with tritium (³H) and evaluated using a human NAAA cell pellet binding assay, leading to the identification of a lead scaffold. Based on this scaffold, a library of 96 fluorinated analogues was designed and evaluated in silico using CNS PET MPO scoring. Guided by these results, three leading fluorinated analogues were selected and

synthesized via 6-, 6-, and 4-step synthetic routes, with overall yields of 2%, 5%, and 15%, respectively. Their *in vitro* binding affinities toward human NAAA were subsequently evaluated, yielding IC_{50} values of 0.67, 3.57, and 17.04 μ M, with corresponding plasma protein binding rates of 36.55%, 20.42%, and 5.54%, respectively. Analogue with the highest binding affinity was taken forward for preparation as a PET radiotracer candidate and efforts towards its preparation will be presented.

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P 06

Therapeutic Efficacy of the GD2-Targeted Molecular Radiotherapy ^{177}Lu -Dinutuximab-beta in Neuroblastoma

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Neuroblastoma is the most common extracranial solid tumour of childhood, with around half of patients categorised as high-risk – who have a 3-year event-free survival of roughly 50% [1]. Hence, new treatments are crucial for this patient population. Molecular radiotherapy using [^{131}I]mIBG is an established clinical treatment of neuroblastoma. However, target expression and clinical outcomes can be variable. An alternative target is GD2 – which is over-expressed on neuroblastomas – and a clinical antibody targeting GD2, Dinutuximab-beta, has improved survival in patients [2]. We previously developed an antibody-targeted molecular radiotherapy against GD2, using ^{177}Lu -labelled Dinutuximab-Beta (^{177}Lu -DB). We then demonstrated specific binding of ^{177}Lu -DB to GD2-positive neuroblastoma tumour cells in vitro and in vivo. In this work, we assessed the therapeutic efficacy and safety profile of ^{177}Lu -DB in mice bearing human neuroblastoma LAN-1 xenografts. ^{177}Lu -DB treatment was given at three different radioactivity doses (13.3, 8.8 and 4.5 MBq) and compared with an untargeted antibody (^{177}Lu -Rituximab, 8.8 MBq), non-radiolabelled DB and vehicle control. To confirm tumour uptake of ^{177}Lu -DB on study, SPECT-CT was performed on mice given 13.3 MBq at 96 h p.i. (**Figure 1A**). ROI analysis confirmed high tumour uptake of $28.1 \pm 4\%$ ID/g Tumour measurements and mouse survival were measured up to 160 d post-treatment. Over this time-period, 55% of mice receiving ^{177}Lu -DB (13.3 MBq) survived with complete primary tumour regression (**Figures 1B and 1C**). The median survival for ^{177}Lu -DB (8.8 and 4.5 MBq) was 117 days and 84 days respectively, whereas the ^{177}Lu -Rituximab (8.8 MBq) and vehicle control had a survival of 26 d and 13 d respectively (**Figures 1D and 1E**). Additionally, the ex vivo biodistribution of ^{177}Lu -DB between 1h-240h in tumour-free mice was measured for human dosimetry estimates. Finally, blood samples taken pre- and post-treatment were used to assess blood toxicity, which will also be presented. In conclusion, ^{177}Lu -DB demonstrated a strong, GD2-specific, anti-tumour effect in the LAN-1 neuroblastoma model.

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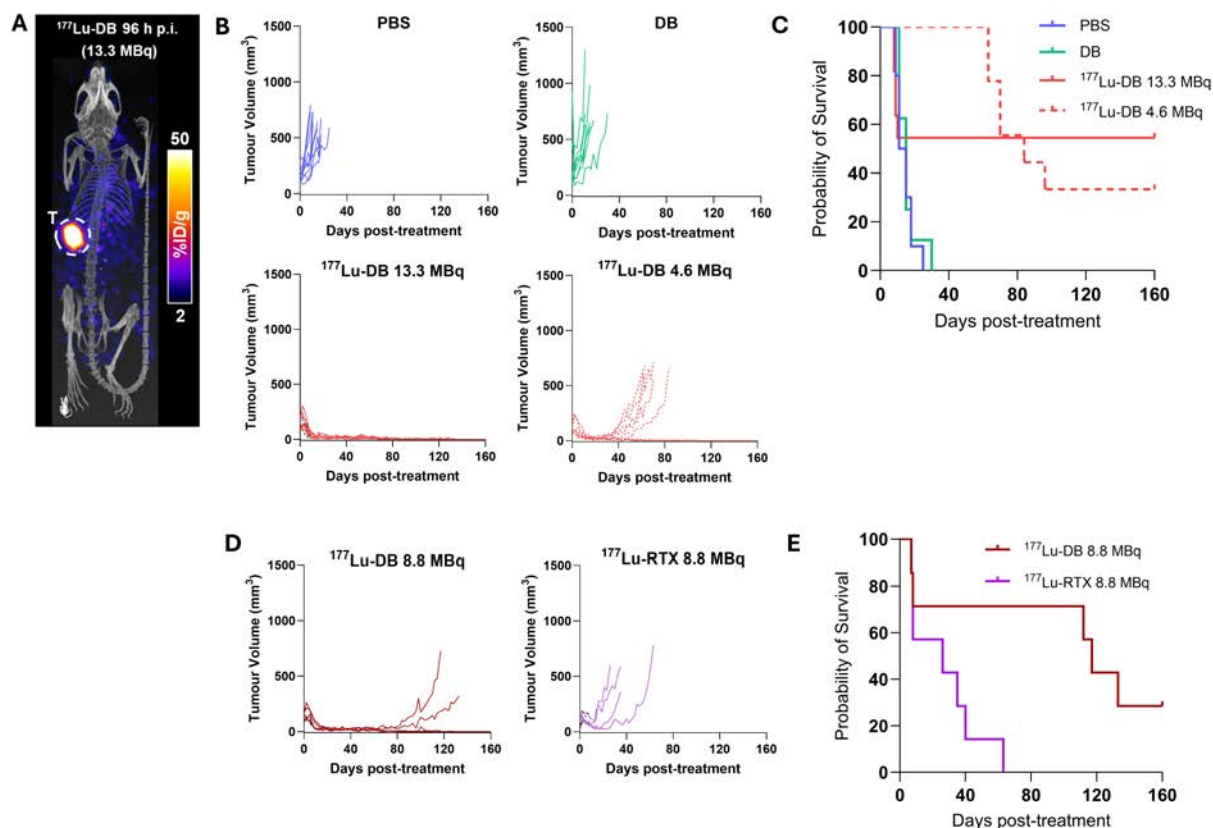


Figure 1. (A) In vivo SPECT-CT maximum intensity projection (MIP) images showing the tumour uptake of ^{177}Lu -DB (13.3 MBq, 40 μg mAb) 96 h after intravenous (i.v.) injection in mice bearing LAN-1 xenografts. (B) Tumour growth over time post-treatment for the PBS vehicle control, DB (non-radiolabelled) and ^{177}Lu -DB (13.3 MBq and 4.5 MBq). (C) Kaplan-Meier plot showing survival over time post-treatment for the PBS vehicle control, DB (non-radiolabelled) and ^{177}Lu -DB (13.3 MBq and 4.5 MBq). Median survival was 13 d, 15 d for PBS and DB respectively, and 84 d for ^{177}Lu -DB (4.5 MBq). 55% of mice receiving ^{177}Lu -DB (13.3 MBq) survived with complete primary tumour regression up to 160 d. (D) Tumour growth over time post treatment for ^{177}Lu -DB and non-targeted control antibody ^{177}Lu -Rituximab (^{177}Lu -RTX). 8.8 MBq dose for each. (E) Kaplan-Meier plot showing survival over time post-treatment ^{177}Lu -DB and non-targeted control antibody ^{177}Lu -Rituximab (^{177}Lu -RTX). 8.8 MBq dose for each. Median survival was 117 d and 26 d respectively.

P 07**Supramolecular Radiotheranostics in Non-Small Cell Lung Cancer**RA Lewis^{1,*}, DM Bäcker², A Barber², JP Holland² and TH Witney¹¹School of Biomedical Engineering and Imaging Sciences, King's College London, St. Thomas' Hospital, London, UK; ²University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057, Zurich, Switzerland

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Patients with non-small cell lung cancer (NSCLC) have poor outcomes, highlighting the need for novel targeted therapies [1]. Epidermal growth factor receptor (EGFR) is overexpressed in 40–80% of NSCLC patients and is therefore an attractive therapeutic target [2]. Antibody-based radiotheranostics against EGFR have shown promise; however, conventionally radiolabelled antibodies exhibit unfavourable pharmacokinetics, leading to off-target radiation exposure and a limited therapeutic window [3]. Supramolecular chemistry, including rotaxane-based platforms, offers a non-covalent approach to fine-tune immunoPET pharmacokinetics [4]. Here, we evaluate a rotaxane-based radiotheranostic platform with the EGFR-targeting antibody cetuximab in NSCLC models.

Methods. Cetuximab was conjugated to rotaxanes, 401 or 403 (**Figure 1A**) or DFO via lysine labelling and radiolabelled with zirconium-89 to generate [⁸⁹Zr]Zr-DFO-cetuximab, [⁸⁹Zr]Zr-401-cetuximab and [⁸⁹Zr]Zr-403-cetuximab [5]. Radiolabelling efficiency was assessed by size-exclusion HPLC, with immunoreactivity evaluated in EGFR-high A549 cells. Biodistribution and PET/CT imaging were assessed in the chickCAM model 24 h post-injection. The platform was evaluated in subcutaneous A549 tumour-bearing BALB/c nu/nu mice, with tumour uptake and pharmacokinetics assessed up to 144 h following intravenous administration of 20 µg radioimmunoconjugate. Data were analysed by unpaired, 1-tailed t-test.

[⁸⁹Zr]Zr-DFO-cetuximab, [⁸⁹Zr]Zr-401-cetuximab and [⁸⁹Zr]Zr-403-cetuximab had radiochemical conversions of 75%, 88% and 90%, respectively (**Figure 1B**). [⁸⁹Zr]Zr-DFO-cetuximab demonstrated the highest immunoreactivity (97±22.7%), with marginally lower values observed for [⁸⁹Zr]Zr-401-cetuximab (79±0.76%) and [⁸⁹Zr]Zr-403-cetuximab (80±0.59%; **Figure 1C**). Compounds were subsequently evaluated in the chickCAM. Imaging showed predominant liver and kidney accumulation of the radiolabelled rotaxane, versus systemic distribution of the radioimmunoconjugate (**Figure 1D**). In a subcutaneous NSCLC in vivo model, tumour uptake at 144 h post-administration increased from 10.9±1.3% AA/g for [⁸⁹Zr]Zr-DFO-cetuximab (n=6) to 15.3±3.4% AA/g for [⁸⁹Zr]Zr-401-cetuximab (p=0.021, n=6) and 17.4±6.1% AA/g for [⁸⁹Zr]Zr-403-cetuximab (p=0.043, n=6) (**Figure 1E**). Despite increased tumour uptake with rotaxane-based radioimmunoconjugates, pharmacokinetic profiles were comparable. Rotaxane-based drugs demonstrated significantly higher tumour-to-blood ratios compared to control, with ratios of 2.4±0.38 vs 3.4±0.60 (p=0.018, n=6) for [⁸⁹Zr]Zr-401-cetuximab and 2.4±0.38 vs 4.22±0.45 (p<0.0001, n=6) for [⁸⁹Zr]Zr-403-cetuximab (**Figure 1F**).

Rotaxane-based radioimmunoconjugates had enhanced tumour uptake, potentially reducing the blood-pool background that can obscure lung tumour imaging with covalently labelled antibodies. Enhanced tumour uptake supports evaluation of rotaxane-based therapeutics.

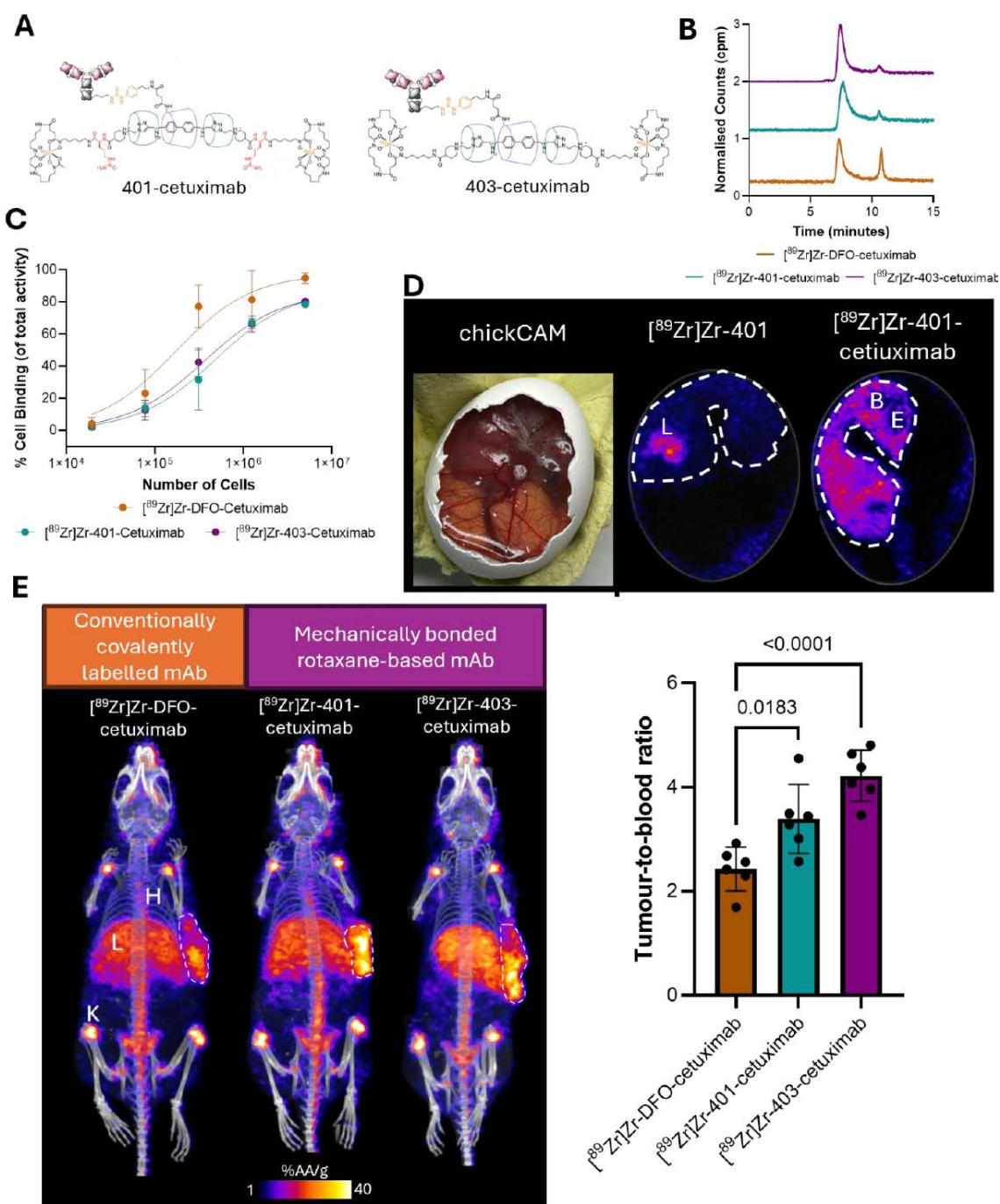


Figure 1. Comparison of conventionally covalently radiolabelled monoclonal antibodies with a supramolecular approach. (A) Rotaxane structures, 401 and 403 conjugated to cetuximab by lysine labelling making 401-cetuximab (left) and 403-cetuximab (right). (B) Size-exclusion HPLC traces of crude reactions of $[^{89}\text{Zr}]\text{Zr}$ -DFO-cetuximab (bottom), $[^{89}\text{Zr}]\text{Zr}$ -401-cetuximab (middle), and $[^{89}\text{Zr}]\text{Zr}$ -403-cetuximab (top). (C) Immunoreactivity assay in EGFR-high A549 cells. (D) Representative coronal PET/CT images of a fertilised chicken embryo 24 h post-injection with $[^{89}\text{Zr}]\text{Zr}$ -401 (middle) and $[^{89}\text{Zr}]\text{Zr}$ -401-cetuximab (right). L=liver, E=eye, B= brain. (E) Representative maximum-intensity-projection (MIP) PET/CT images of BALB/c nu/nu mice bearing subcutaneous A549 tumours at 144 h post-injection of 20 μg of $[^{89}\text{Zr}]\text{Zr}$ -DFO-cetuximab, $[^{89}\text{Zr}]\text{Zr}$ -401-cetuximab,

and [^{89}Zr]Zr-403-cetuximab; tumour is depicted by the white dotted line. L=liver, H=heart, K=knee. (F) Tumour-to-blood ratios at 144 h post-injection of [^{89}Zr]Zr-DFO-cetuximab, [^{89}Zr]Zr-401-cetuximab, and [^{89}Zr]Zr-403-cetuximab.

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P 08

Combination of anti-GD2 Molecular Radiotherapy and $\gamma\delta$ -T-cells for the Enhanced Treatment of Childhood Bone Cancers

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Ewing sarcoma (EwS) and osteosarcoma (OS) are the most common bone cancers in young people. The 5-year survival rates for non-metastatic EwS and OS are 70% and 66%, respectively - which decrease to below 30% with detectable metastases. Standard treatment of both involves surgery and chemotherapy with external beam radiotherapy only being efficacious for EwS. These treatment plans have remained unchanged for 30 years, thus stagnating 5-year survival rates. $\gamma\delta$ T-cells have previously been shown killing both EwS and OS. These are innate immune cells, safe to administer in the allogeneic setting. Like Natural Killer cells, $\gamma\delta$ T-cells are capable of antibody dependent cellular cytotoxicity (ADCC). Therefore, $\gamma\delta$ T-cells can target EwS and OS by labelling them with Dinutuximab beta (DB), an antibody which targets upregulated ganglioside GD2 on their surfaces. This project aims to investigate combining low-dose molecular radiotherapy and $\gamma\delta$ T-cells as a synergistic treatment for metastatic EwS and OS. $\gamma\delta$ T-cells respond to stressed or damaged cells - we hypothesise that a beta or alpha particle emitting molecular radiotherapy will therefore additionally sensitise targets (**Figure 1**).

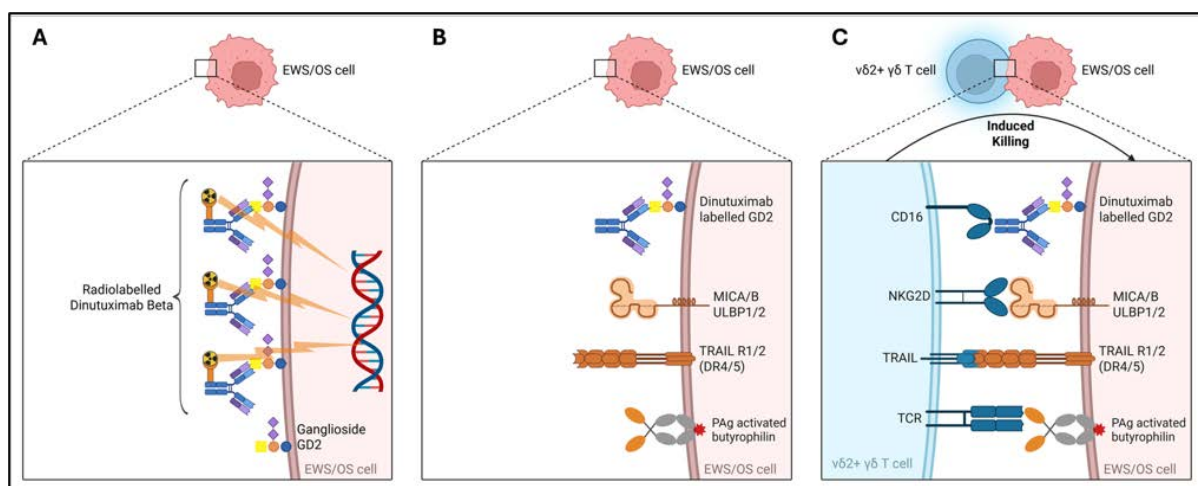


Figure 1. Treatment strategy for EWS and OS pursued in the presented project

We have successfully conjugated DB and radiolabelled with ¹⁷⁷Lu (a beta particle emitter) with radiochemical yields > 95%. Post-conjugation, binding was validated using LAN-1, a high GD2 expressing cell line. Binding of radiolabelled antibody was checked against a range of GD2 expressing OS cell lines. Currently, an investigation into using conjugated DB for ADCC assay of $\gamma\delta$ T-cells against OS cells is in progress. In the future, we will use live cell imaging

to quantify DNA damage and cell stress induced by molecular radiotherapy in 2D and 3D models. These results will guide us to the optimal timepoint for the addition of $\gamma\delta$ T-cells. The potential synergy of molecular radiotherapy and immunotherapy may open avenues for the detection and subsequent treatment of other metastatic cancers.

P 09

Development of Targeted PET-PDT Theranostics for Breast Cancer

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There is a distinct need for new therapies that overcome treatment resistance and selectively target specific breast cancer subtypes to improve patient outcomes [1,2]. Photodynamic therapy (PDT) is an effective cancer treatment that bypasses many traditional resistance mechanisms through light-activated cytotoxicity [3,4]. Theragnostic approaches enable both diagnosis and treatment to 'see and treat' cancers, allowing real-time monitoring of therapeutic response using positron emission tomography (PET) to support personalised treatment strategies [5,6].

Development of a novel multifunctional drug incorporating a PET radiotracer, a photosensitiser for PDT, and monoclonal antibodies for breast cancer. The therapeutic efficacy and selectivity of this agent will be evaluated in vitro and in vivo to maximise cancer cell killing while minimising damage to healthy tissue. The drug will be synthesised and assessed for photochemical properties, stability, and radiolabelling efficiency. It will be tested in breast cancer cell lines to evaluate uptake and light-induced toxicity. Preclinical studies will follow in tumour-bearing CD-1/nude mice using fibre-optic laser delivery, with PET imaging to monitor biodistribution and treatment response. Optimisation of a targeted theragnostic treatment demonstrating significant breast tumour reduction with minimal off-target toxicity (**Figure 1**).

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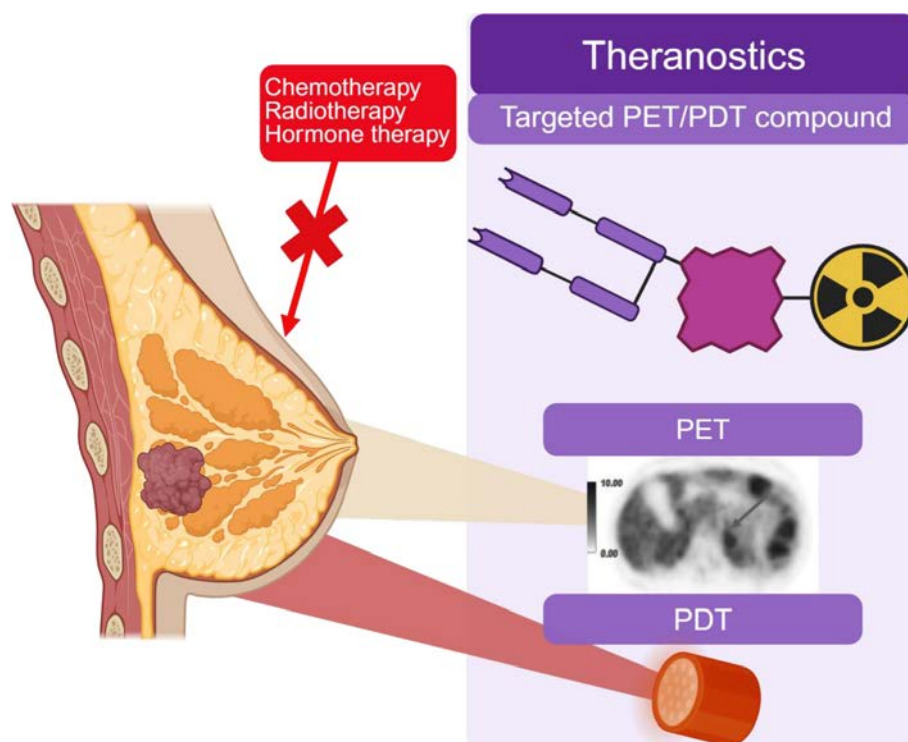


Figure 1. Strategy for the development of a theragnostic treatment of breast cancer

P 10

Bridging Imaging and Tissue Biology: The Significance of Ex-Vivo Analysis for Biomarker Characterisation

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Ex-vivo tissue analysis is an essential approach for characterising disease-associated biomarkers, signalling pathways, and immune responses, ranging from single-marker immunohistochemistry to multiplex spatial imaging. CXCR4 and ACKR3 are seven-transmembrane G-protein-coupled receptors that are frequently dysregulated in cancer and have emerged as potential diagnostic and therapeutic targets. Although CXCR4-targeted imaging using gallium-68-labelled Pentixafor has demonstrated clinical utility, the expression patterns and relationship between CXCR4 and ACKR3 across aggressive solid tumours remain incompletely characterised. Comprehensive ex-vivo tissue profiling of these receptors may therefore provide important insights into their biological significance and potential utility as therapeutic targets.

CXCR4 and ACKR3 expression was evaluated in human multiple myeloma xenograft tumours and orthotopic lung tumours derived from murine models, as well as in murine liver, normal human liver, and human liver cancer tissue blocks obtained from external suppliers. Immunohistochemical analysis was performed using single-marker staining and co-staining protocols to characterise the expression and distribution of CXCR4 and ACKR3 within the tissues.

Murine liver and human liver cancer tissues demonstrated higher CXCR4 staining compared with normal human liver tissue. Multiple myeloma xenograft tumours also showed strong CXCR4 staining in specific tumour regions. In contrast, orthotopic lung tumour sections exhibited more intense ACKR3 staining compared with CXCR4, indicating differential receptor expression across tumour types and tissues (**Figure 1**).

Differential expression of CXCR4 and ACKR3 across tumour and organ types highlights the potential molecular importance of these receptors in tissue-specific tumour biology and disease progression. Further investigation of CXCR4 and ACKR3 expression across a broader range of organs, tumour types, and disease stages may provide valuable insights into their potential applications in early disease detection, patient stratification, and therapeutic targeting of aggressive tumours.

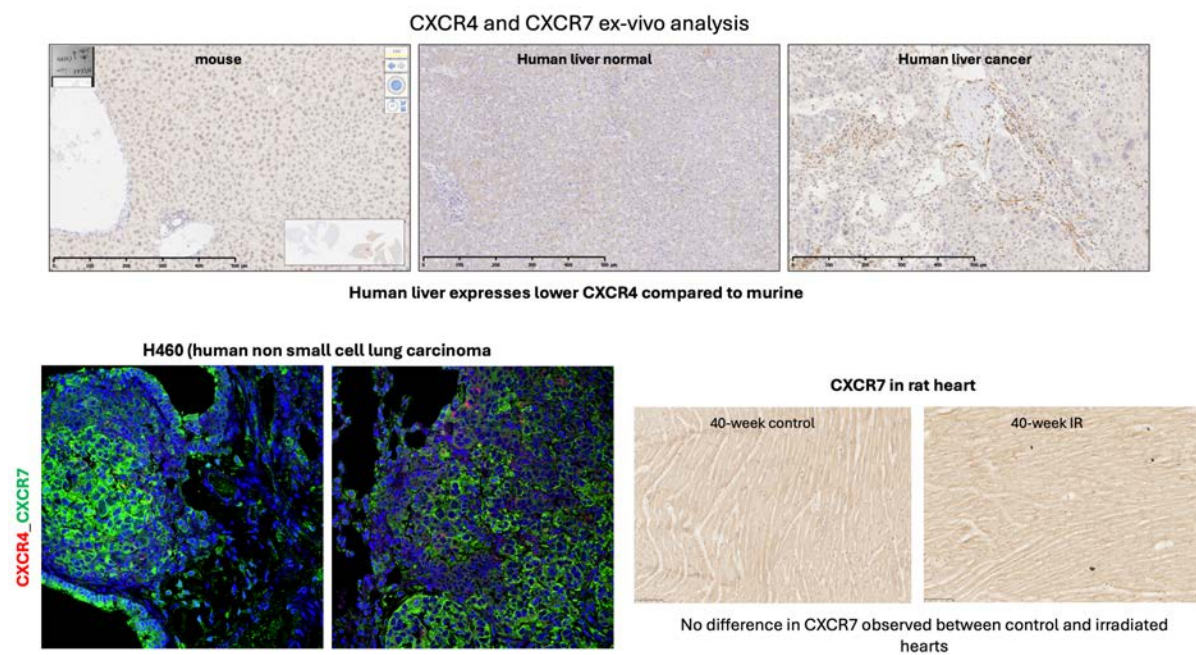


Figure 1. Expression of CXCR4 and CXCR7 in human and rodent tissues

P 11

Bicycle® Radionuclide Conjugates (BRCs): A Versatile Platform for Developing Novel Radiopharmaceuticals Against a Broad Portfolio of Oncology Targets

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The expanding landscape of precision oncology demands novel imaging agents capable of providing rapid, high-contrast visualisation of clinically relevant targets. While antibodies and small molecules have limitations in pharmacokinetics or specificity, Bicycle® peptides represent a unique, clinically validated modality. Their small size allows for rapid tissue penetration and fast renal clearance, while their large binding surface area enables antibody-like affinity and specificity to target. Bicycle Radionuclide Conjugates (BRCs) are designed to utilise Bicycle targeting vectors to deliver radioisotopes for both diagnostic imaging and targeted radiotherapy (theranostics). This preclinical work showcases the power and versatility of the Bicycle® platform to rapidly generate potent and specific binders suitable for radiopharmaceutical development against a diverse range of cancer specific targets [1,2].

Utilising a proprietary phage display platform, potent binders were identified against multiple targets. Lead candidates were optimised for affinity, stability, and pharmacokinetic properties. Optimised peptides were functionalised with chelators for site-specific radiolabelling with isotopes such as Ga-68. These BRCs were evaluated in vitro for binding affinity and specificity, and in vivo via PET imaging as well as ex vivo biodistribution studies in relevant tumour xenograft models.

This robust discovery and optimisation process will be demonstrated across a portfolio of high-value oncology targets, including MT1-MMP, EphA2 and Nectin-4. Preclinical data for a range of BRCs showing high affinity, target specificity and favourable biodistribution properties will be presented. For example, nanomolar binding affinity MT1-MMP targeting BRCs- (4.1 - 9.8 nM) which allow high tumour uptake in vivo (12 - 25 %ID/g) while maintaining low kidney levels (5.8 – 6.1 %ID/g).

The Bicycle® platform has proven to be a robust, scalable, and highly effective engine for the discovery and development of novel radiopharmaceuticals. The unique properties of bicyclic peptides translate directly into superior agents with rapid, high-contrast tumour visualisation and favourable tumour to background profiles. This has the potential to deliver a pipeline of best-in-class PET diagnostics and radiotherapeutics across a range of high value cancer targets to address critical unmet needs in patient stratification and treatment monitoring across a breadth of cancer indications.

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P 12

Dynamic [^{11}C]Leucine PET/MRI for Imaging Tumour Protein Synthesis in Colorectal Cancer

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Protein synthesis supports tumour growth and survival [1]. Non-invasive imaging of this process could reveal differences in tumour biology and provide a means of assessing therapeutic response [2]. Non-invasive imaging of these processes could provide insights into tumour biology. We investigated whether dynamic [^{11}C]leucine PET/MRI distinguishes colorectal tumours with different protein synthesis activity associated with loss of the amino-acid transporter SLC7A5.

Subcutaneous tumours were established using two colorectal cancer organoid models derived from genetically engineered mice: VillinCreER Apcfl/fl KrasG12D/+ (AK) and VillinCreER Apcfl/fl KrasG12D/+ Slc7a5fl/fl (AKS) (**Figure 1A**). Loss of SLC7A5 in this model reduces protein synthesis [3]. Mice [n = 4 per group] underwent 120-minute dynamic PET imaging following intravenous administration of 26–32 MBq [^{11}C]leucine, with MRI for anatomical localisation. Complementary [^{13}C]leucine tracing measured labelled leucine in plasma and tumours and its incorporation into protein (at 15, 30, 60, 90 and 120 min; n = 3 per group). RNA in situ hybridisation, immunohistochemistry and proteomic analyses characterised differences between tumour models.

Dynamic [^{11}C]leucine PET/MRI demonstrated sustained tumour tracer retention, with higher uptake in AK than AKS tumours (**Figures 1B and 1C**). Tumour SUV_{mean} was [AK value $\pm$ variability versus AKS value $\pm$ variability over the specified time window] (P = 0.0285, two-way ANOVA). [^{13}C]leucine tracing characterised the time course of labelled leucine availability in plasma and AK tumours (**Figure 1H**). Protein-incorporation analysis showed greater [^{13}C]leucine incorporation in AK than AKS tumours [n = 4 per group; BH-FDR-adjusted P < 0.001] (**Figure 1E**). Together, sustained PET retention and independent evidence of leucine incorporation into protein support a contribution of protein synthesis to the tumour PET signal. AK tumours showed higher Slc7a5, Sesn2 and Axin RNA expression (**Figures 1F to 1H**), whereas AKS tumours showed increased CD45-positive immune-cell infiltration (**Figure 1I**).

Dynamic [^{11}C]leucine PET/MRI differentiates colorectal tumours with distinct protein synthesis activity. Sustained tracer retention, supported by independent evidence of leucine incorporation into protein, supports its potential for non-invasive imaging of tumour protein synthesis. These findings provide a basis for evaluating its ability to monitor treatment-induced changes in protein synthesis.

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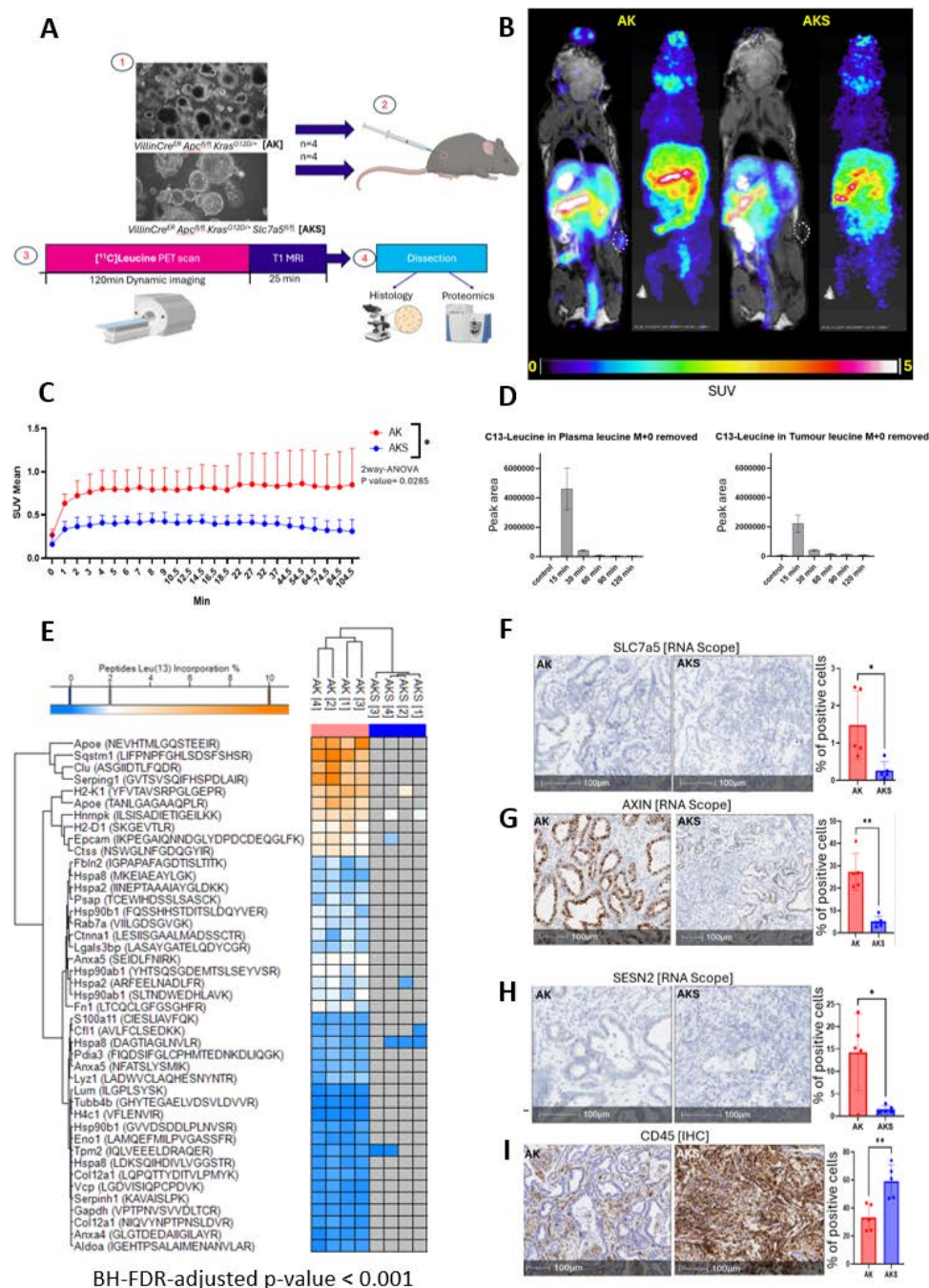


Figure 1. [^{13}C]Leucine PET/MRI in AK and AKS tumours

P 13

Development of an In Vivo Traceable Orthotopic Rat Breast Cancer Model for Longitudinal Assessment of EGFR-targeted Therapy

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Breast cancer is a highly heterogeneous disease affecting 11.6% of women globally [1]. Tumour associated antigens, epidermal growth factor receptor (EGFR/ERBB1) and human epidermal growth factor receptor 2 (HER2/ERBB2), are overexpressed in approximately 15-30% [2]. Breast cancer patient cohorts with tumours co-expressing EGFR and HER2 are associated with poorer overall and distant metastasis-free survival [3]. Development of an orthotopic immunocompetent disease-relevant and in vivo traceable rat model would be highly beneficial for effective evaluation of targeted therapies relevant to this patient cohort.

We conducted lentiviral engineering of MTLn3, with rat sodium iodide symporter fused to green fluorescent protein (rtNIS-GFP) (**Figure 1A**). In vitro reporter stability and function was assessed via flow cytometry, immunoblot, and radiotracer uptake. Immunocompetent female Fischer 344 rats were challenged with MTLn3.rtNIS-GFP cells. Tumour establishment and EGFR-targeted therapy efficacy was assessed via calliper measurements and longitudinal SPECT/CT imaging, with subsequent ex vivo immunohistochemistry analyses.

Results. Immunoblotting data confirmed bulk expressions of rtNIS-GFP (**Figure 1B**), alongside weekly flow cytometry showing reporter purity (**Figure 1C**). Immunofluorescence staining demonstrated correct reporter colocalization (**Figure 1D**), which is the prerequisite of rtNIS-GFP function. MTLn3.rtNIS-GFP cells demonstrated efficient [^{99m}Tc]TcO₄⁻ uptake, which was inhibited by the competitive NIS substrate, perchlorate, confirming functionality (**Figure 1E**). Importantly, MTLn3.rtNIS-GFP cells retained growth kinetics as the proliferation pattern was unaltered following reporter engineering (**Figure 1F**). Immunocompetent female Fischer 344 rats challenged with MTLn3.rtNIS-GFP into the inguinal mammary fat pad, successfully formed tumours confirmed by calliper measurement (**Figure 1G**), and a baseline SPECT/CT scan at day 14 (**Figure 1H**). Calliper measurement suggested MTLn3.rtNIS-GFP tumours were susceptible to erlotinib, which was supported by a lower live tumour volume in SPECT/CT (**Figure 1I**). Ex vivo analyses demonstrated a significant reduction in phospho-

EGFR expression in erlotinib-treated tumours compared to control (**Figure 1J**), confirming effective inhibition of EGFR signalling and pharmacodynamic target engagement in vivo.

We successfully established a syngeneic orthotopic rat breast cancer platform that recapitulated key biological features of a breast cancer patient cohort with poor prognosis. This toolbox is useful for longitudinal assessment and spatiotemporal monitoring of targeted therapeutic responses in a clinically relevant setting.

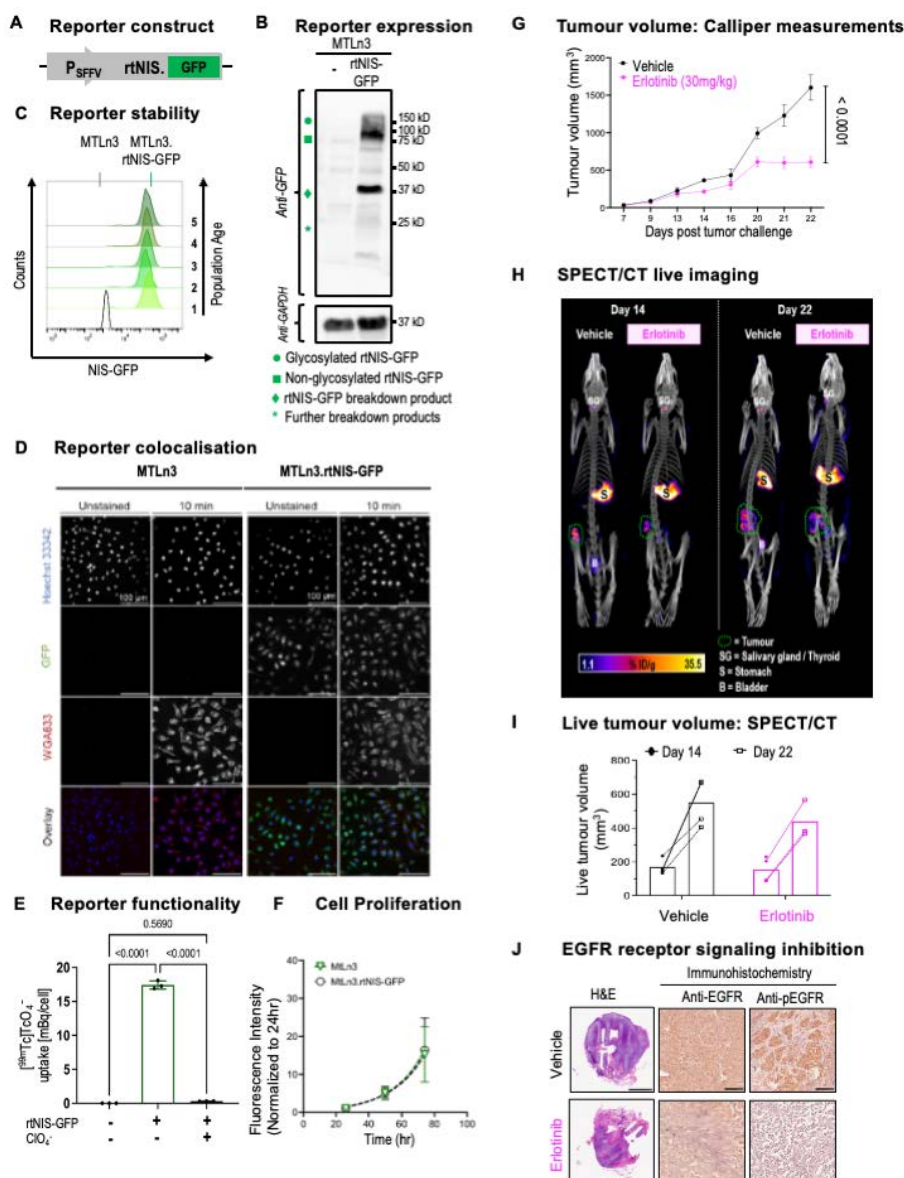


Figure 1. Preclinical characterisation of the developed orthotopic rat breast cancer model

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P 14

Development of an In Vivo Traceable Triple-negative Breast Cancer Model to Study the Radiation-induced Abscopal Effect

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Triple-negative breast cancer (TNBC) often spreads and patients have poor prognosis and few treatments are available due to its lack of targeted therapeutic options [1,2]. Radiotherapy is a potent immune response modulator contributing to local (i.e. irradiated) and systemic tumour control ('abscopal effect'/AE) [3]. AE is not fully understood mechanistically with the impact of primary tumour mass irradiation also remaining elusive [4,5], thus limiting clinical exploitation. Reliable 3D quantification of tumour burden in immunocompetent TNBC models will benefit AE research.

We engineered in vivo traceable murine E0771 TNBC cells with the mouse sodium iodide symporter (NIS) fused to a fluorescent protein (**Figure 1A**). GFP supported cell line generation while NIS unlocked whole-body radionuclide tomography. Traceable cells were confirmed for reporter expression and functionality. Unilateral or bilateral orthotopic tumours were established in female immunocompetent C57BL/6 mice. Palpable tumours received 2x 8 Gy by targeted external beam radiotherapy (tEBRT), with [^{99m}Tc]TcO $_4^-$ afforded NIS-SPECT/CT scans flanking tEBRT. Excised tumours were subjected to ex vivo fluorescence imaging and histology to evaluate intratumoral immune cell infiltration.

E0771.NIS-GFP cells showed reporter purity (**Figure 1B**) and correct colocalisation (**Figure 1C**), took up [^{99m}Tc]TcO $_4^-$ (**Figure 1D**), and exhibited similar growth patterns to parental cells (**Figure 1E**). Baseline SPECT/CT of palpable tumours permitted quantification of live tumours in the mammary fat pads (**Figure 1F** left). After tEBRT, all irradiated tumours shrank, as reflected by initial calliper measurement (**Figure 1H** left). Notably, bilateral non-irradiated tumours displayed a growth delay when compared to the unilateral non-irradiated group, requiring more time to reach ~200mm ^{3} volume (**Figure 1H** right). Post-irradiation SPECT/CT showed irradiation led to tumour necrosis (**Figure 1F** right). Moreover, bilateral non-irradiated tumours resulted in a lower radiotracer uptake than those non-irradiated tumours established unilaterally (**Figure 1G**). Ex vivo immunofluorescence staining revealed higher CD8 $^+$ T cell infiltration into the bilateral non-irradiated tumour core compared to the unilateral non-irradiated tumours (**Figure 1I**), suggesting systemic T cell-mediated effects contributing to the observed AE.

We demonstrated in vivo tumour burden tracking and quantification to reveal AE in this tEBRT setting. This study unleashes future AE investigations in conjugation with immunotherapy and debulking surgery.

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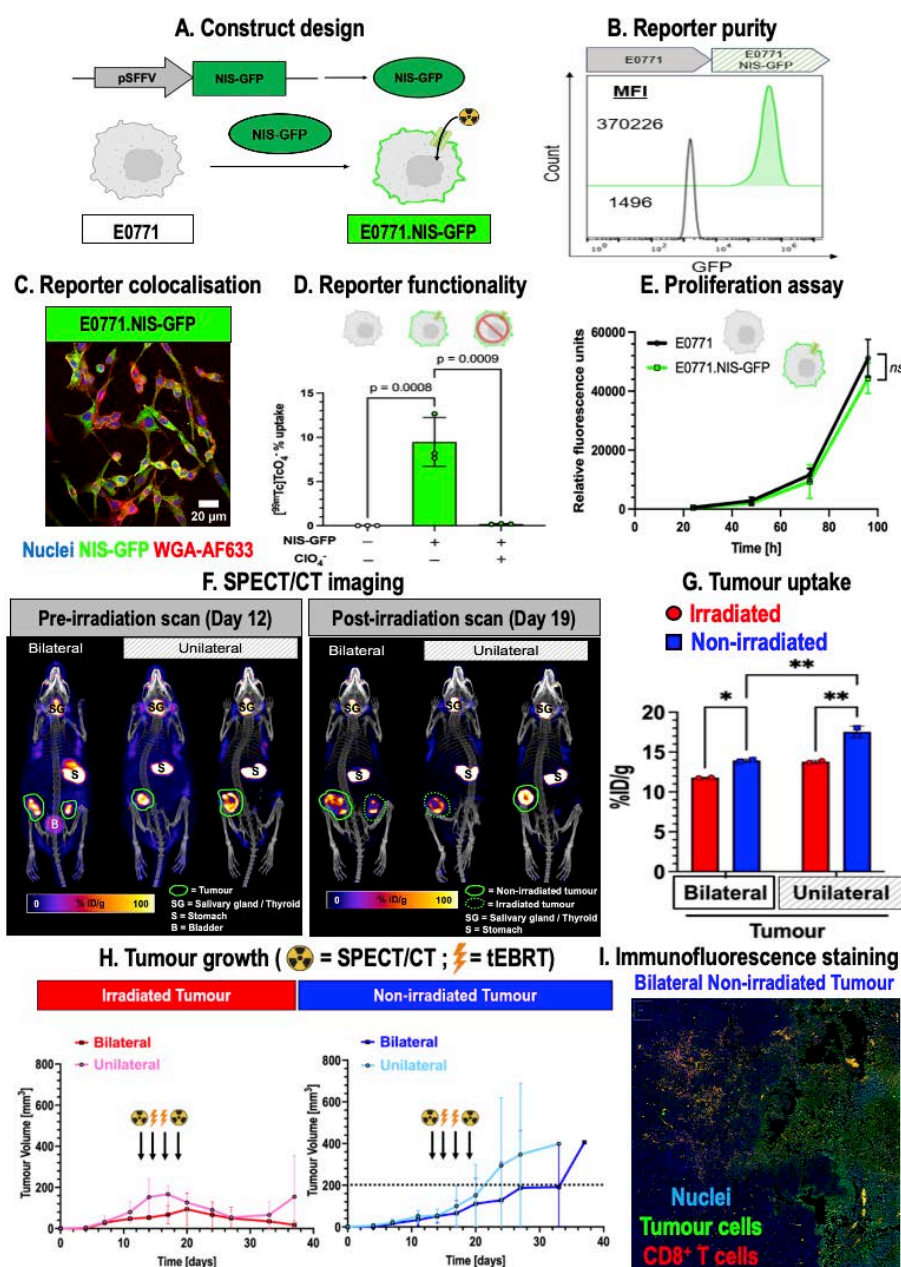


Figure 1. Preclinical characterisation of the developed triple-negative rat breast cancer model

P 15

Bridged Cyclam Scaffolds for the Development of CXCR4-Targeted Theranostic Agents

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Chemokine receptors play essential roles in numerous physiological and pathological processes and are implicated in a broad range of diseases, including inflammatory disorders, HIV infection, and cancer. Among them, CXCR4 has emerged as a promising therapeutic and imaging target due to its overexpression in various malignancies and its association with tumour progression, aggressive disease phenotypes, and poor prognosis [1]. The development of CXCR4-targeted agents therefore offers opportunities to improve tumour detection, characterize disease biology, and support patient stratification and personalized treatment strategies. Our group has previously developed a library of configurationally restricted tetraazamacrocyclic compounds and their Cu(II), Zn(II), and Ni(II) complexes, demonstrating high CXCR4 affinity both in vitro and in vivo [2]. Notably, these compounds can be directly radiolabelled with copper radionuclides, including ⁶⁴Cu for positron emission tomography (PET) imaging and ⁶⁷Cu for single-photon emission computed tomography (SPECT) imaging and targeted radionuclide therapy [3]. However, the introduction of additional chelating units, such as DOTA, could broaden the range of compatible radiometals and further expand the theranostic potential of this molecular platform. In this study, we report the synthesis and characterization of a new series of CXCR4-targeted, configurationally restricted bis-macrocyclic derivatives based on a bridged cyclam scaffold. A range of different functionalization strategies were investigated to enable further molecular diversification, including N- and C-functionalization of one macrocycle and aryl functionalization of the bridging unit. These modifications aim to provide versatile platforms for the incorporation of additional chelating moieties while preserving CXCR4-targeting properties. Preliminary in vitro and in vivo evaluations have yielded encouraging results, with high CXCR4 affinity and substantial tumour uptake observed in xenograft models. Further investigations are ongoing to validate these findings and establish the impact of structural modifications on biological performance. Overall, this modular bis-macrocyclic approach represents a promising strategy for developing CXCR4-targeted radiopharmaceuticals with potential applications in molecular imaging and targeted radionuclide therapy.

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P 16

Novel CXCR4- and ACKR3-targeted Theranostic Agents for PET/SPECT Imaging and Radionuclide Therapy in Cancer

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The chemokine receptors CXCR4 and ACKR3 (previously CXCR7) have been shown to be overexpressed in many different cancers [1] and usually indicate a poor prognosis. Many imaging agents and therapeutic compounds have been studied for the targeting of CXCR4; however, few exist for ACKR3. Given the coordinated nature of CXCR4 and ACKR3 driven biochemical processes, targeting both receptors simultaneously could yield more favourable therapeutic outcomes.

[⁶⁴Cu]CuCB-Bicyclam is a high-affinity radiotracer for the PET imaging of CXCR4-overexpressing tumours [2,3]. Copper(II), zinc(II), nickel(II), and manganese(II) analogues of this compound have been developed in our research group and show high affinity for CXCR4 and ACKR3. Radiolabelling selected candidates with ⁶⁴Cu (PET) and ⁶⁷Cu (SPECT and radionuclide therapy) has shown promising preliminary results for both imaging and therapy of these chemokine receptors.

A novel high-affinity CXCR4/ACKR3-targeted agent, Cu₃H₂TrisCB-Cyclam, has been developed, and its derivatives have been successfully radiolabelled with ⁶⁴Cu and ⁶⁷Cu. SPECT/CT imaging of the ⁶⁷Cu derivative, [⁶⁷Cu]SJA999, showed significant tumour uptake in animals implanted with CXCR4- and ACKR3-overexpressing xenografts, with high tumour uptake maintained at least 3 days post-injection. Specificity of [⁶⁷Cu]SJA999 for CXCR4 was confirmed in blocking studies using the high-affinity CXCR4 antagonist, Cu₂CB-Bicyclam (**Figure 1**).

Future work will focus on extending the in vivo validation of our novel dual CXCR4/ACKR3 inhibitor to better understand how this derivative interacts with the two chemokine receptors of interest. Further evaluation will be carried out across a range of tumour models, including those with heterogeneous CXCR4 and ACKR3 expression, as well as in radiopharmaceutical therapy experiments.

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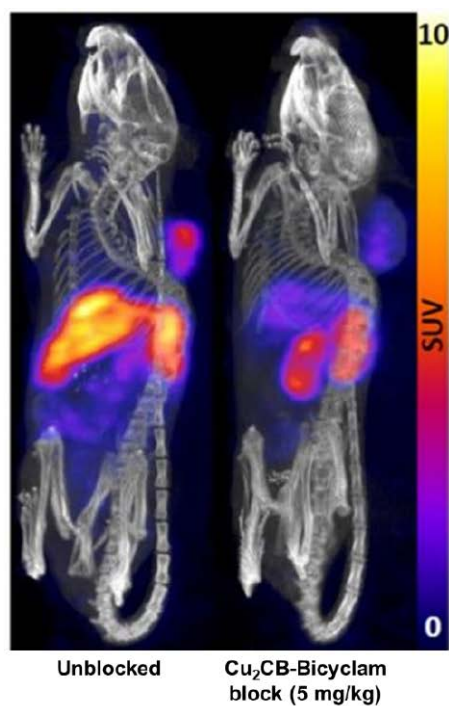


Figure 1. Representative 30 min SPECT/CT scan of ⁶⁷Cu-radiolabelled CXCR4/ACKR3 dual inhibitor in U87-CXCR4 tumour-bearing mice, unblocked (left) and blocked with 5 mg/kg (i.p.) of Cu₂CB-Bicyclam (right), imaged at 3 d post-injection

P 17

Novel Double Imprinted Nanoparticles Targeting xCT for Enhanced Drug Delivery in NSCLC

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Non-small cell lung cancer (NSCLC) remains a common cancer with poor prognosis, particularly in treatment resistant disease. Approximately 30% of NSCLC tumours overexpress the cystine/glutamate antiporter SLC7A11 (xCT) conferring aggressive disease but also presenting a therapeutic target [1,2]. Here, we report novel double-imprinted polymeric nanoMIPs (NMs) imprinted with doxorubicin (DOX) and targeting extracellular domain 3 of xCT for selective drug delivery (**Figure 1A**). We assessed their physicochemical properties, in vitro targeting in NSCLC cell lines, and in vivo localisation. NMs loaded with fluorescein (FNM) or DOX (DNM) were synthesised using peptide-templated polymerisation on epitope-derivatised glass beads and purified using a solid-phase extraction [3,4]. NMs were assessed for size, morphology, and binding affinity through DLS, SEM, and SPR. NMs were then assessed in xCT high (H460) and xCT low (H1299) NSCLC cell lines for cytotoxicity (MTT), using flow cytometry and confocal imaging. Finally, whole-body distribution was assessed through confocal imaging after intratumoral or intravenous administration of NMs in subcutaneous H460 mice models. DNM binding specificity for xCT was ~50-fold higher than for the unspecific epitope (32 ± 53 vs 1570 ± 2473 nM) and ~3-fold higher between DOX and daunorubicin (122 ± 8 vs 344 ± 420 nM; **Figures 1B and 1C**). There was differential FNM binding between H460 and H1299 cells ($757 \pm 56\%$ vs $277 \pm 24\%$ change in median fluorescence intensity; **Figure 1D**), indicating specificity for xCT. DOX-loaded DNM decreased cell viability to $51 \pm 24\%$ and $66 \pm 5\%$ in H460 and H1299 cells, respectively (**Figure 1E**). Finally, we demonstrated localization of FNM to the tumour, liver, and spleen 24 hours after intravenous administration (**Figures 1F and 1G**). We report the successful synthesis and purification of NMs imprinted for xCT and DOX, with homogenous morphology and low nM binding affinity. In NSCLC lines, we confirmed xCT-specific binding and localisation, biocompatibility, targeted cytotoxicity, and NM internalization including in vivo tumour delivery. Future studies will include evaluating treatment efficacy and epitope mapping to improve xCT binding affinity. To conclude, this is the first-in-class xCT-targeted dual-imprinted nanoparticles that offer a robust and innovative approach to address treatment resistant NSCLC through molecularly guided drug delivery.

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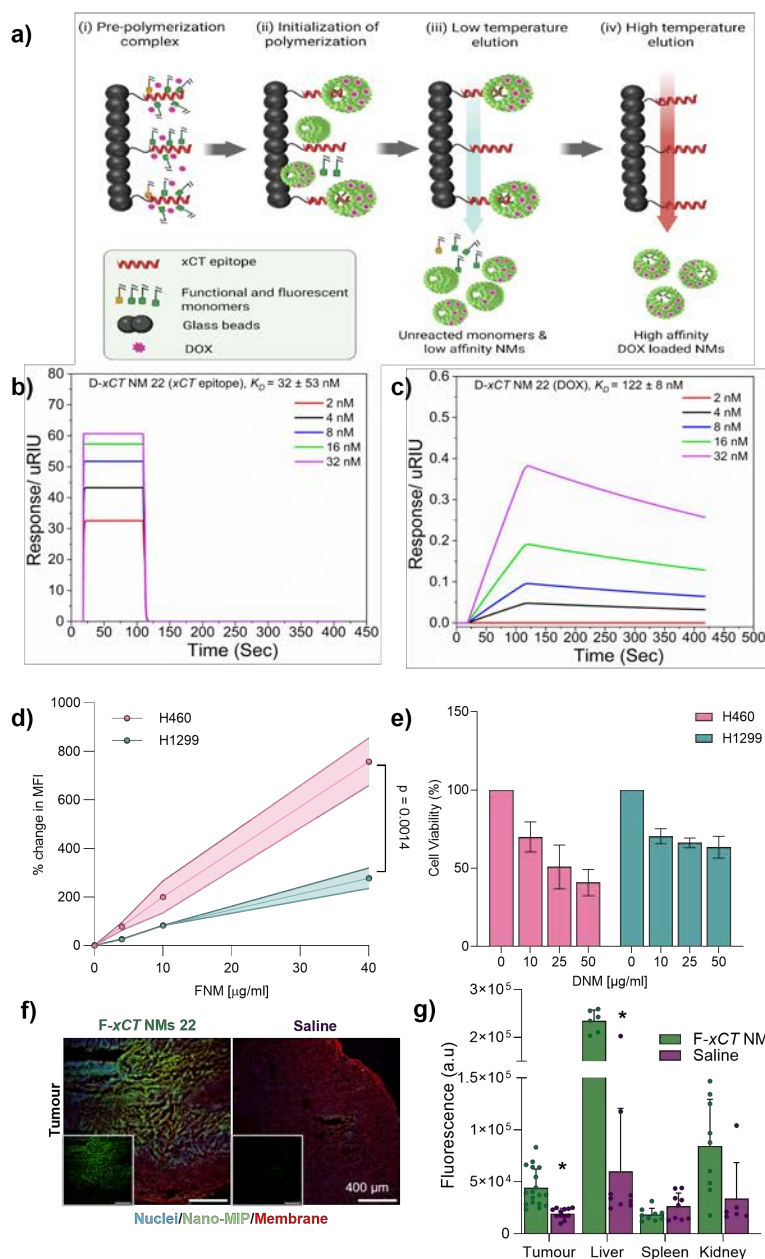


Figure 1. (A) Schematic of synthesis of double-imprinted NMs: the primary template is the xCT epitope of 22 AA conjugated on silanized beads, whilst DOX acts as secondary template in solution. B-C) SPR sensorgrams used to determine binding affinities of DNM to the xCT epitope (B) and doxorubicin (C). (D) Calculated median fluorescence intensity (MFI) change between H460 and H1299 cells normalised to mean baseline fluorescence ($n=3$). (E) MTT viability analysis of DNM in H460 and H1299 cells with 24h treatment. (F) Representative confocal images of H460 subcutaneous tumour 24 hours after intravenous F-xCT NMs 22 (5 mg/kg) administration or saline (vehicle). (G) Image quantification of F-xCT NMs 22 fluorescence per cell ($p < 0.05$) ($n=18$ tumour, $n=9$ organ images). Scale bar: 400 μ m. Error bars: SEM

P 18

Multimodal Imaging of Heart-Brain Alterations in a Mouse Model of Diabetes

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Type 2 diabetes (T2D) is a chronic metabolic disorder affecting multiple organs, including the heart and brain. However, the concurrent metabolic alterations across these organs remain incompletely understood. Preclinical multimodal imaging enables non-invasive assessment of organ-specific pathophysiology in vivo [1]. This study aimed to establish a multimodal imaging platform to characterize diabetes-induced cardiac and cerebral metabolic alterations using [^{18}F]-FDG-PET/CT to quantify glucose uptake.

Male C57BL/6J mice (8 weeks old) were assigned to T2D (n=10) or control (n=5) groups. T2D mice were fed a high-fat diet (HFD; 60 kcal% fat) and subsequently administered low-dose streptozotocin (STZ; 50 mg/kg/day, intraperitoneally) for 5 days after 4 weeks of HFD feeding. Controls received standard chow and sodium citrate buffer. Blood glucose was measured weekly. At week 11, PET/CT (Mediso NanoScan) was performed 60 min after intravenous [^{18}F]-FDG administration (5 MBq) to quantify cardiac and cerebral glucose uptake.

Fasting blood glucose was significantly elevated in T2D mice from 1 week after STZ administration and remained elevated throughout the study. [^{18}F]-FDG-PET demonstrated significantly lower glucose uptake in both the brain (SUVmax: 1.03 ± 0.23 vs. 1.91 ± 0.78) and heart (SUVmax: 3.40 ± 1.04 vs. 12.96 ± 3.03) of T2D mice compared with controls, demonstrating substantial metabolic alterations in both organs (**Figure 1**).

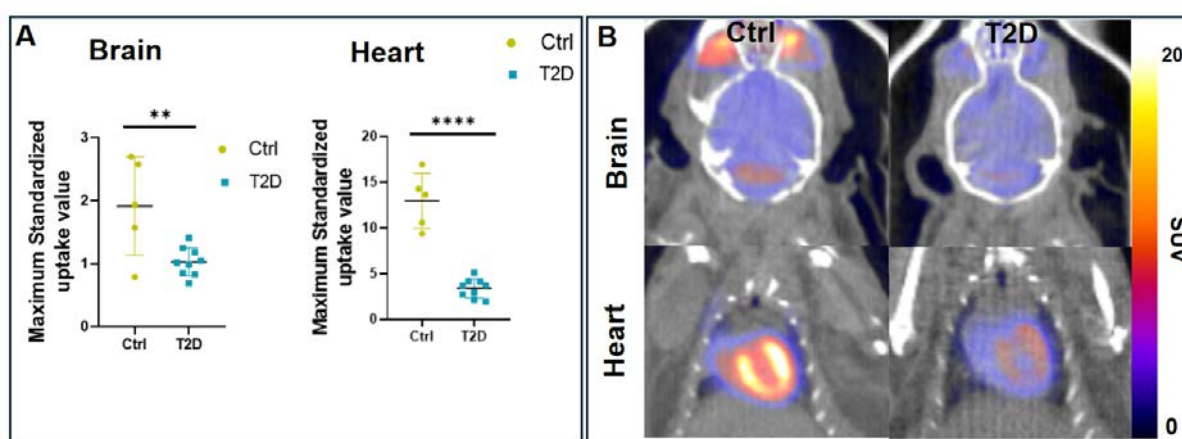


Figure 1. Cardiac and cerebral evaluation by ^{18}F -FDG PET-CT in control and T2D mice; (A) SUVmax from ^{18}F -FDG PET-CT; (B) Representative PET-CT images of ^{18}F -FDG uptake

[^{18}F]-FDG-PET/CT identified parallel cardiac and cerebral metabolic alterations in experimental T2D. This non-invasive imaging approach provides a sensitive platform for characterizing multi-organ metabolic consequences of diabetes and may facilitate longitudinal

evaluation of therapeutic interventions aimed at preserving cardiac and cerebral metabolic integrity.

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P 19

Automated Radiosynthesis of [^{18}F]Fluoromethylcarnitine for PET Imaging of Fatty Acid Metabolism

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Carnitine-mediated transport of fatty acids into mitochondria is essential for fatty acid oxidation (FAO). Cellular carnitine uptake is mediated by organic cation transporter novel type 2 (OCTN2), which in turn regulates mitochondrial energy metabolism and contributes to metabolic adaptation in cancer. [^{18}F]Fluoromethylcarnitine ([^{18}F]FMC) holds potential to non-invasively interrogate these metabolic processes in vivo, however, reliable automated production methods are required for routine [^{18}F]FMC synthesis. Here, we developed the first automated radiosynthesis of [^{18}F]FMC.

[^{18}F]FMC was successfully synthesised using the automated cassette-based synthesis on the Trasis AllinOneTM. Implementation of a 2-step one-pot radiosynthetic approach simplified the automated procedure and reduced intermediate steps. Optimisation of the SPE purification, including strategic cartridge positioning and washing procedures, improved RCY from $0.03 \pm 0.01\%$ ($n = 2$) to $7.8 \pm 1.4\%$ ($n = 5$) and reduced residual impurity peaks observed by radio-HPLC (**Figure 1**). The optimised synthesis afforded [^{18}F]FMC with $>99\%$ RCP, as determined by radio-HPLC (**Figure 2**). The final product was formulated in saline to ensure biocompatibility and obtained at a radioactive concentration of 30 - 70 MBq/mL, which remained stable for up to 4h, supporting its suitability for subsequent biological applications including metabolic imaging studies. Preliminary in vitro studies evaluating OCTN2-mediated uptake of [^{18}F]FMC, including comparison between automated and manually synthesised [^{18}F]FMC in a model of triple-negative breast cancer, are currently ongoing.

Robust automated radiosynthesis of [^{18}F]FMC was successfully developed, yielding the [^{18}F]FMC with high RCP. The cassette-based synthesis enabled reproducible production of [^{18}F]FMC and was sufficient for large-scale preclinical studies from a single synthesis batch. This automated synthesis platform supports further investigation of [^{18}F]FMC as a PET tracer to assess carnitine-mediated uptake and fatty acid metabolism across a range of diseases, providing a step towards an automated process suitable for GMP production of [^{18}F]FMC.

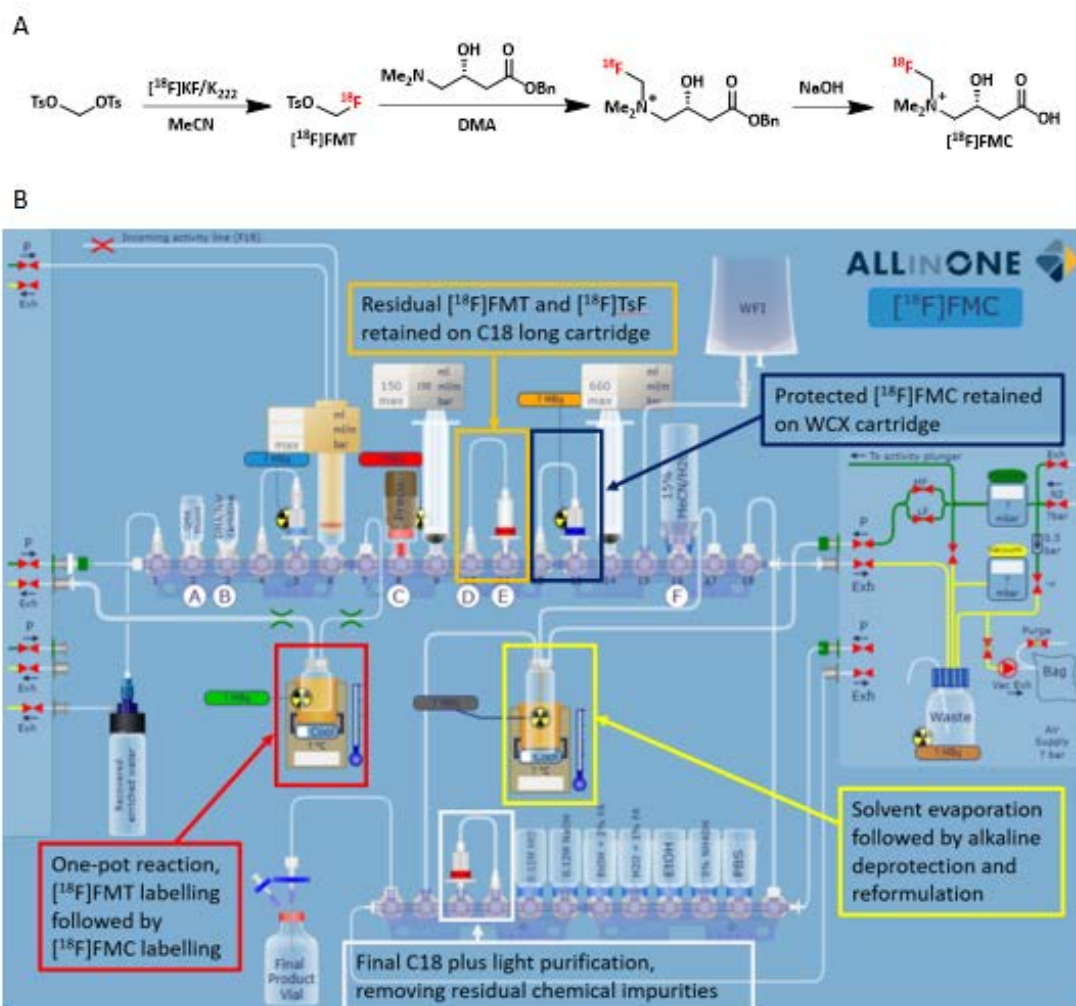


Figure 1. Automated radiosynthesis of [^{18}F]FMC on the Trasis AllinOneTM. (A) Radiosynthetic scheme. (B) Schematic representation of the [^{18}F]FMC cassette design: a 30-valve system incorporating five manifolds enabled one-pot radiosynthesis, SPE purification, solvent evaporation, deprotection, and final saline formulation. Strategic positioning of the C18 long and WCX cartridges selectively trapping reaction intermediates and removing residual impurities during purification. Highlighted boxes indicate the key steps.

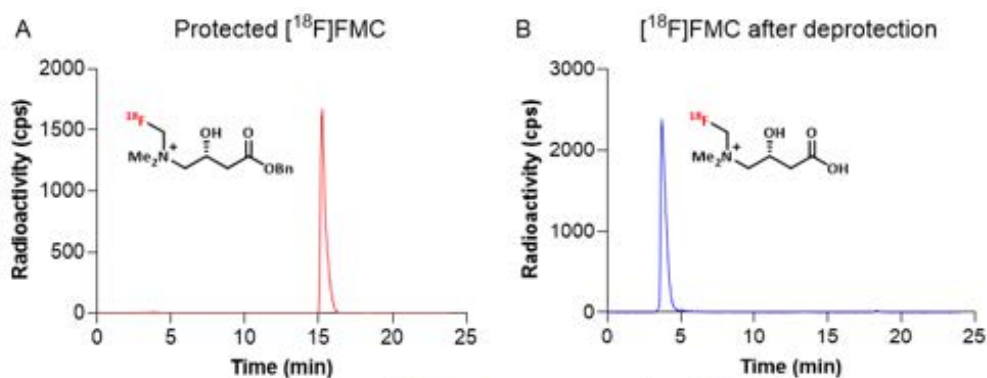


Figure 2. Radio-HPLC chromatogram of (A) protected [^{18}F]FMC intermediate and (B) final [^{18}F]FMC following deprotection. During optimisation process, the protected [^{18}F]FMC intermediate was collected and analysed prior to deprotection to confirm the absence of radiochemical impurities as residual [^{18}F]FMT and [^{18}F]TsF could also undergo deprotection, contributing to impurities in the final product.

P 20

Tracking Micronutrient Dynamics In Vivo Using PET: Imaging the Fate of Essential Metal Ions

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The emergence of total-body PET offers new opportunities to investigate fundamental biological questions concerning trafficking and in vivo fate of essential micronutrients in health and disease states. Manganese, copper and zinc are essential dietary metal ions, each with a PET-compatible radioisotope that enables the non-invasive tracking of their absorption, distribution and excretion [1]. However, previous studies have predominantly investigated radiometal biodistribution following intravenous administration. Here, we investigate their fate following oral administration by gavage, providing insight into gastrointestinal absorption and subsequent systemic distribution via the dietary route.

⁵²Mn, ⁶⁴Cu and ⁶²Zn were produced by cyclotron irradiation and purified to yield [⁵²Mn]MnCl₂, [⁶⁴Cu]CuCl₂ and [⁶²Zn]Zn-citrate, respectively. Each radiometal was administered by oral gavage to healthy male and female BALB/c mice without prior fasting, to reflect nutrient handling under typical feeding conditions. Radiometal distribution was monitored longitudinally by PET/CT at multiple time points, with faeces collected at each time point. At 48 h post-administration, mice were culled and tissues collected to assess radiometal uptake by gamma counting and endogenous Cu, Zn, Mn and Fe levels by ICP-MS.

PET imaging highlighted markedly different gastrointestinal absorption and distribution of the three radiometals. ⁵²Mn exhibited minimal intestinal absorption, limiting image quantification. ⁶²Zn showed the highest absorption and tissue distribution, although image quantification was hindered by lower image resolution. In contrast, ⁶⁴Cu combined good absorption with favourable image quality, enabling quantification across all time points. Following intestinal absorption, copper accumulated initially in the liver, with distribution in other tissues becoming visible at later timepoints. Tissue ⁶⁴Cu uptake correlated with endogenous Cu levels ($r=0.619$). However, heart and brain deviated from the global trend, showing higher levels of endogenous Cu than predicted from ⁶⁴Cu uptake and highlighting organ-specific copper handling. Interestingly, a similar linear relationship was observed between ⁶²Zn uptake and endogenous Zn levels across most organs, with bone as an exception.

This study demonstrates the potential of PET for non-invasive, longitudinal tracking of orally administered essential metal ions for at least 48 h. The linear relationship between ⁶⁴Cu/⁶²Zn uptake and endogenous Cu/Zn levels shows that PET can provide insights into tissue-specific metal homeostasis without the need for biopsy. With the advent of total-body PET, this approach could be translated to humans to investigate metal ions physiology in health and disease.

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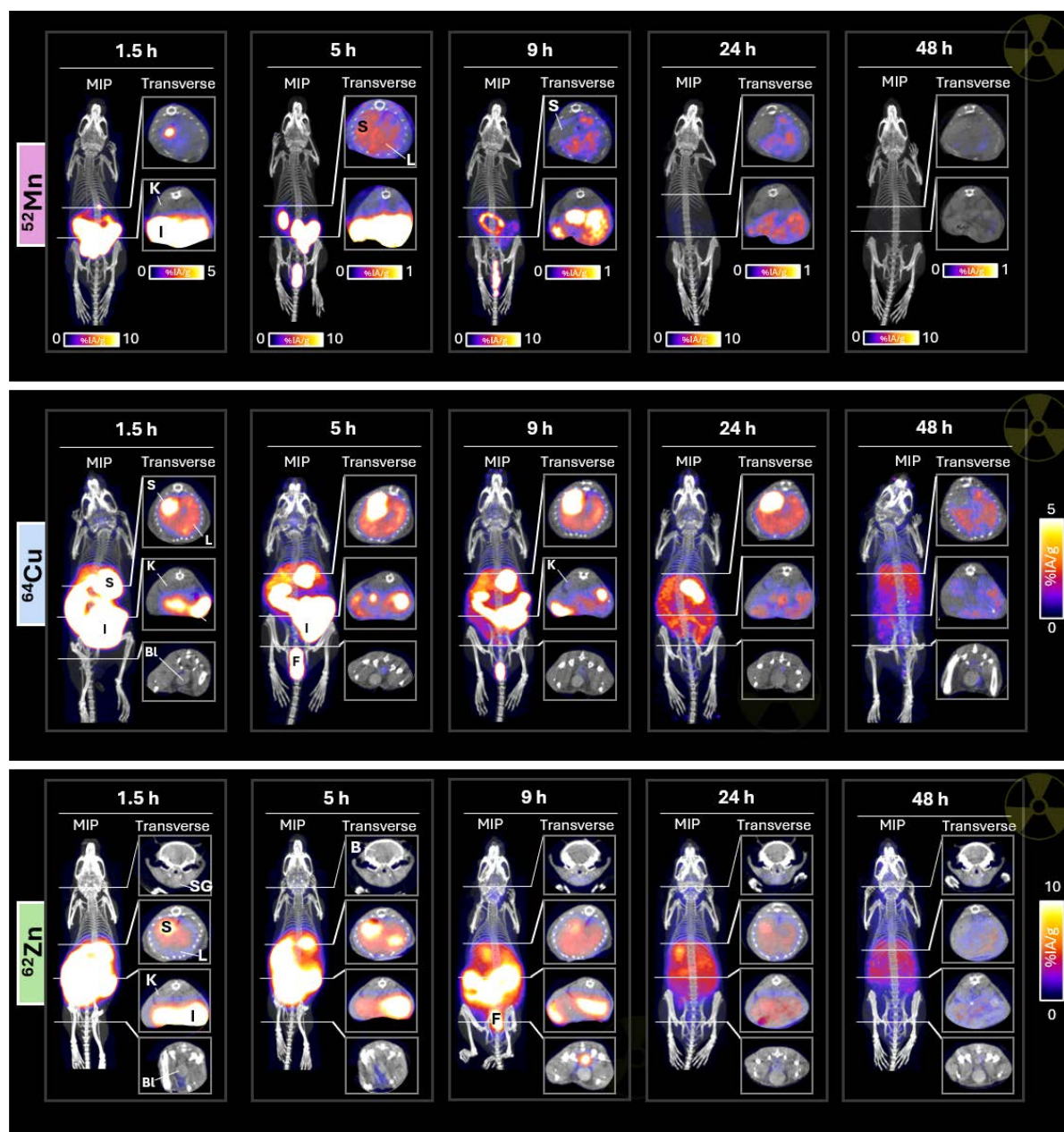


Figure 1. Longitudinal PET/CT imaging of orally administered ^{52}Mn , ^{64}Cu and ^{62}Zn in mice. Representative maximum intensity projection (MIP) and transverse PET/CT images acquired at 1.5, 5, 9, 24 and 48 h following oral administration of ^{52}Mn (top), ^{64}Cu (middle) and ^{62}Zn (bottom). The images illustrate the temporal changes in radiotracer distribution across the body and selected organs. Colour scales indicate radiotracer activity expressed as percentage injected activity per gram (%IA/g). Distinct uptake and clearance patterns are observed for the three radiometals over the 48 h imaging period

P 21

⁶⁸Gallium-Labelled Siderophores for Microbial Infection Imaging

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Antimicrobial resistance creates an urgent need for pathogen-specific diagnostic tools that can guide antimicrobial treatment and monitor response. Nuclear imaging can localise suspected infection but cannot reliably distinguish microbial infection from sterile inflammation or malignancy. [¹⁸F]FDG PET/CT detects increased glucose metabolism and primarily reflects the host response rather than the pathogen. Because current imaging approaches lack infection specificity, efforts have increasingly focused on pathogen-targeted radiopharmaceuticals¹. Siderophore-based radiotracers exploit conserved microbial iron-uptake pathways and may enable highly selective imaging of bacterial and fungal infections, including pathogens that utilise xenosiderophores [1-3]. Working with King's Health Partners, UKHSA and King's colleagues, we are developing siderophore-based PET imaging agents [4-6].

Siderophores are high-affinity iron-chelating molecules used by bacteria and fungi for iron acquisition. Because gallium(III) shares key coordination properties with iron(III), gallium-68 (⁶⁸Ga) can replace iron within siderophore complexes, enabling PET imaging of microbial iron uptake. We developed radiosynthesis methods and in vitro uptake assays to screen natural and synthetic ⁶⁸Ga-labelled siderophores against clinically relevant bacterial and fungal pathogens, including *Aspergillus fumigatus*, *Candida albicans*, and *Candidozyma auris*. Tracers were evaluated under different growth conditions, in *A. fumigatus* biofilms and in an *A. fumigatus*-A549 human lung epithelial co-culture model. A lead radiotracer was assessed by ex vivo biodistribution and whole-body PET/CT imaging.

In vitro screening identified [⁶⁸Ga]Ga-schizokinen as a promising bacteria-specific radiotracer. The tracer demonstrated favourable tissue distribution and clearance in ex vivo biodistribution studies and whole-body PET/CT imaging. Screening of fungal pathogens identified three candidate tracers. One candidate demonstrated uptake in the presence of clinically used antifungals and immunosuppressants and also retained uptake in biofilm and co-culture

models. This tracer is currently being evaluated in animal infection models with support from the MRC Gap Fund.

^{68}Ga -labelled siderophores offer a promising strategy for pathogen-specific infection imaging. Having demonstrated microbial uptake and specificity *in vitro*, the lead radiotracers warrant evaluation of *in vivo* targeting and their ability to distinguish infection from sterile inflammation. Further studies will assess safety, biodistribution, radiation dosimetry and GMP-compliant manufacturing to support first-in-human study. Siderophore PET could support earlier diagnosis, treatment-response monitoring and targeted antimicrobial use, particularly in immunocompromised patients.

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P 22

Phantom Characterisation of Quantification Limits in ^{18}F and $^{99\text{m}}\text{Tc}$ Dual-isotope SPECT Imaging

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Dual-isotope SPECT imaging with ^{18}F (511 keV) and $^{99\text{m}}\text{Tc}$ (140 keV) could enable simultaneous visualisation of distinct molecular processes, but high-energy photons from ^{18}F contribute substantial downscatter into the $^{99\text{m}}\text{Tc}$ detection window, complicating absolute $^{99\text{m}}\text{Tc}$ quantification and source reconstruction. We quantified the ^{18}F -to- $^{99\text{m}}\text{Tc}$ downscatter fraction and assessed the performance of a SPECT/CT system in imaging mixtures of both radionuclides, with the aim of optimising the activity ratio for quantitative dual-isotope imaging.

Energy spectra were extracted from SPECT data of phantoms containing individual or mixed sources of ^{18}F or $^{99\text{m}}\text{Tc}$ across mixing ratios of 10:1 to 1:1 activity concentration, using a dedicated high-energy collimator (APT56) for detection up to 600 keV gamma radiation. Downscatter contribution from ^{18}F into the $^{99\text{m}}\text{Tc}$ window ($140\text{ keV} \pm 10\%$) was quantified from energy spectra. Spatial resolution, volume sensitivity, and recovery coefficients were derived from phantom data. Crosstalk was evaluated before and after scatter correction across the full range of activity ratios, and ^{18}F count-rate linearity was assessed over 0.1-167 MBq.

The mean downscatter fraction from ^{18}F into the $^{99\text{m}}\text{Tc}$ detection window was $18.7 \pm 0.2\%$, whereas reverse crosstalk from $^{99\text{m}}\text{Tc}$ into the 511 keV detection window was negligible ($<1\%$). The smallest rod visible on reconstructed hot rod phantom images was 1.2 mm and 1.0 mm for ^{18}F and $^{99\text{m}}\text{Tc}$ respectively, providing a measure of achievable spatial resolution with the imaging system. Absolute activity was well recovered for individual sources of ^{18}F and $^{99\text{m}}\text{Tc}$ ($\pm 3\%$ and $\pm 0.1\%$ difference from dose-calibrator measurements respectively). Bias in $^{99\text{m}}\text{Tc}$ quantification was kept to within $\pm 1.1\%$ up to mixing ratios of 7:1 ^{18}F : $^{99\text{m}}\text{Tc}$ when scatter correction was applied but exceeded 10% if scatter correction was disabled for mixing ratios of 2:1 or higher. Impact from ^{18}F -to- $^{99\text{m}}\text{Tc}$ downscatter was reduced to negligible levels under optimal image reconstruction settings, demonstrating the potential of quantitative multi-tracer imaging on the studied SPECT device (**Figure 1**).

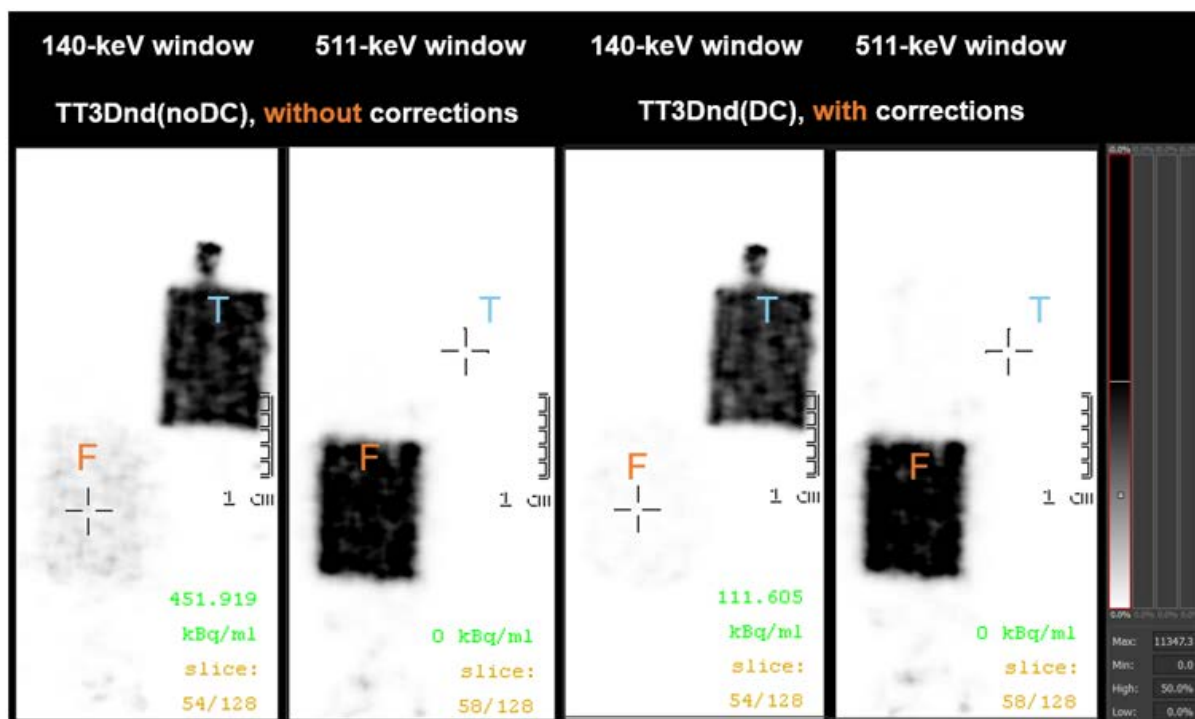


Figure 1. Phantoms containing ^{18}F and $^{99\text{m}}\text{Tc}$, respectively, as measured on a SPECT/CT system, without (left) and with (right) scatter corrections

P 23

Preclinical PET Imaging of Radionuclide-Dependent Image Quality

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Historically, the performance characteristics of PET scanners under established standards are recommended to be performed using ^{18}F [1]. As a wider range of radionuclides become available with PET imaging applications, image quality assessments may reveal limitations or advantages in radionuclide choice. This work compares the NEMA NU4 image quality metrics of ^{18}F , ^{52}Mn , ^{64}Cu and ^{68}Ga , using a commercial preclinical PET system.

NEMA NU4 image quality phantoms were prepared with 3.7 MBq of either ^{18}F , ^{52}Mn , ^{64}Cu or ^{68}Ga . PET data were acquired with the phantom positioned at the centre field of view for 20 minutes and reconstructed as per manufacturer guidelines. Image data were analysed using an in-house developed python script. Edge detection algorithms from line profiles generated over each axis of the image matrix were used to automatically locate and extract region of interest data from the known phantom geometry.

Image uniformity was in good agreement for all radionuclides, with % standard deviations ranging between 9.33 (^{18}F) and 13.46% (^{52}Mn). Spillover ratios were consistent between air and water compartments, and between all radionuclides with the exception of ^{68}Ga , suggestive of good accuracy of correction in PET reconstruction. Spillover in both air and water compartments were markedly higher for ^{68}Ga , attributed to the longer positron range of ^{68}Ga decay. Similarly, the recovery coefficients returned profiles consistent with the radionuclides' respective positron ranges. ^{18}F , ^{52}Mn and ^{64}Cu all showed similarly high contrast recovery from 4 and 5 mm diameter rods (85-92%), with gradual loss of contrast recovery towards 1 mm. The recovery coefficient for ^{68}Ga was consistently poorer for all rod diameters, returning only 60% at 5 mm. Of note, and despite having a similar positron range and lower branching ratio, ^{64}Cu outperformed ^{18}F in this metric.

A range of radionuclides were investigated under NEMA NU4 image quality standards using a preclinical PET scanner. System uniformity was consistent in all assessments. Spillover ratios and recovery coefficients were poorer using ^{68}Ga compared to other investigated radionuclides. These findings have now been reproduced using a clinical long axial field-of-view scanner with additional radionuclide assessments ongoing, including ^{124}I .

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The PNI symposium is a collaborative meeting organised by colleagues from the University of Cambridge, King's College London (KCL) and University College London (UCL).

Please do not hesitate to get in touch throughout the meeting – we are happy to help address any questions you may have.



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